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THE EFFECT OF CYCLING TEMPERATURES ON ELECTROLYTE BALANCE IN SKELETAL MUSCLE AND PLASMA OF RAINBOW TROUT, SALMO GAIRDNERI

by

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FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled The Effect of Cycling Temperatures on Electrolyte Balance in Skeletal Muscle and Plasma of Rainbow Trout, Salmo gairdneri, submitted by Daniel Peter Toews in partial fulfilment for the degree of Master of Science.

ABSTRACT

The physiological effect of cycling temperatures on electrolyte balance in skeletal muscle and plasma of 1½ - year-old rainbow trout (Salmo gairdneri) was studied. The "cycling fish" were maintained in a large tank in which the water temperature cycled from 8°C to 18°C daily, closely simulating daily temperature conditions in several Alberta mountain streams. After 42 days of exposure to the cycle, the fish were sampled at various temperatures. Muscle and plasma samples were analyzed for sodium, potassium, chloride, and water content.

Six other groups of control trout were acclimated for 42 days at six different constant temperatures which coincided with the same temperatures of the cycling fish.

Muscle water content in the cycling fish was significantly lower at 8°C of the cycle, but did not change significantly throughout the rest of the cycle. The control fish showed a significant drop in muscle water content at 16°C and 18°C which corresponded to a rapid growth rate at these temperatures.

Muscle chloride content in the cycling and control fish showed nearly constant levels even though large fluctuations in plasma chloride levels occurred. A reciprocal relationship was found between the muscle sodium and potassium levels in the cycling fish.

Levels of plasma sodium, chloride, and potassium in the cycling fish were similar to the levels in the control fish held at low temperatures. Cycling fish appeared to

be like cold adapted fish, in their water and electrolyte metabolism, which had acclimated to the lower end of the daily temperature cycle.

It was also found that plasma chloride binding occurred in fish acclimated to temperatures of 4°C but not at 12°C. Because it appears that chloride does not distribute itself according to a Donnan distribution, the chloride space and chloride-potassium space cannot serve as valid estimates of extracellular fluid volume. The sodium space in almost all cases was smaller than the chloride space or the chloride-potassium space and was therefore used as an estimate of extracellular fluid volume. In the controls and cycling fish the sodium space decreased significantly with either an increase in holding temperature or an increase in temperature of the cycle.

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"....tous les mécanismes vitaux quelque variés qu'ils soient, n'ont toujours qu'un but, celui de maintenir l'unité des conditions de la vie dans le milieu intérieur."

Claude Bernard (1878)

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INTRODUCTION

Within the past fifteen years much interest has centered on the question of how poikilotherms successfully adjust to large variations in their physical environment. Because it is believed that adaptation to environmental change takes place ultimately at the cellular level, several studies have been carried out to examine the effect of controlled variation of a single parameter of the physical environment, for example temperature, on cell function. Temperature and salinity are the parameters that have received most attention from fish physiologists.

Brett (1956) summarised the work through 1955 on the major thermal requirements of fish. By this time it was known that each species of fish has an upper and lower lethal temperature which could be altered by the acclimation temperature. The actual determinant relationships between lethal temperatures and cellular function have yet to be ellucidated.

Both the rate and the magnitude of temperature change affects physiological acclimatization to temperature in fishes. Cocking (1958) reported that the exposure of the roach, (Rutilus rutilus), to rates of temperature increase greater than .05°C per hour caused increased ammonia excretion, which he suggested was the result of protein catabolism induced by heat shock.

Recently structural and functional changes at the level of the cell, in response to temperature changes, have been

demonstrated. Hochachka and Hayes (1962) reported that brook trout (Salvelinus fontinalis) acclimated to low temperatures utilize different glycolytic pathways than when acclimated to higher temperatures. Freed (1965) found that cold acclimation causes an increase and warm acclimation a decrease in amount of cytochrome oxidase in goldfish muscle. Johnston and Roots (1964) suggested that acclimation involves the ability to control the degree of unsaturation of cellular lipids to maintain a specific liquid-crystalline state of cellular membranes. They also suggest that cold acclimation may release various ions from their binding sites.

Adaptive changes in the central nervous system of fishes, in response to temperature changes, have also been found. Roots and Prosser (1962) showed that the temperature of cold-blockage of a conditioned response in goldfish could be changed by temperature acclimation. Konishi and Hickman (1964) found an increase in nerve conduction velocity and central response time in rainbow trout acclimated to low temperatures and a decrease in response time in trout acclimated to high temperatures. Baslow and Nigrelli (1964) observed changes in brain cholinesterase activity levels in the killifish (Fundulus heteroclitus) in response to experimentally induced thermal stress.

It is generally assumed that the physiological response of a fish to the temperature of the surrounding medium is genetically determined. Stroganov (1956) found that the mosquito fish (Gambusia affinis holobrooki) could be genetically

selected for cold resistance. Ushakov (1964), another Russian worker, studied the thermostability of 279 species of poikilotherms, including several species of fishes. He characterized the thermostability of cells by the intensity of the thermal effect which causes irreversible damage to the protoplasm. He found that 82.5% of the poikilotherms studied from separate populations, living in habitats of different temperatures, did not differ in their thermostability. Ushakov suggests that the absence of differences show that the thermostability of cells and protoplasmic proteins is a conservative species characteristic for the majority of poikilotherms.

Information related to electrolyte changes in an organism is important in a study of thermal acclimation because many properties of cell function are modified by changes in transcellular electrolyte concentrations. Low temperature can affect sodium and potassium conductance in nerve axons (Hodgkins and Keynes 1955) or they can affect the energy dependent sodium-potassium pump which maintains sodium at a low extracellular level (Crescitelli 1957).

Hickman et al. (1964), using two-year-old rainbow trout (Salmo gairdneri), observed various ionic changes following a 10°C temperature drop from 16°C to 6°C over a 17 hour period. Large shifts in sodium and potassium were observed in the skeletal muscle in the first three days of cold exposure, although estimates of the extracellular space volumes were not significantly altered. Both the tissue sodium and potassium decreased during the first three days at the low temperature resulting in an increase in plasma sodium and

potassium. Very slight changes were obtained in electrolyte balance within the first hour of exposure to the cold temperature. Because larger changes take much more time, Hickman et al. suggested that rapid temperature changes, such as those encountered in a daily temperature cycle, would probably produce a much less marked effect on body fluid and electrolyte balance.

More recently, Heinicke and Houston (1965) observed various ionic changes in the goldfish, Carassius auratus, following the heat shock of a 16-18°C temperature change. They noted that heat shock caused a decrease in plasma chloride concentration and cellular phase volume, and an increase in tissue water content and extracellular phase volume. They concluded that heat shock results in the impairment of osmoregulatory capabilities in the goldfish. It has been suggested by other experimenters (Wilkgren 1953; Forster and Berglund 1956) that increased urinary electrolyte loss is a common stress response in fishes.

The physiology of fishes in an alternating temperature regimen has only recently been studied. Lindsey and Ali (1965) studied the effect of an alternating temperature on vertebral count in the medaka (Oryzias latipes). They found that eggs maintained at cycling temperatures showed the highest survival and smallest number of abnormalities in vertebral count when compared to eggs reared at constant temperatures. They suggest that eggs subjected to alternating temperatures become acclimated to a temperature intermediate to the high and low temperature extreme, and therefore do not experience the complete temperature shock from the one temperature extreme to the other.

Heath (1963) compared thermal tolerances in sea-run cutthroat trout (Salmo clarki clarki) exposed to "square-wave" cycled temperature and constant temperature. The cycle length differed between groups of fish (6 hour, 12 hour, 24 hour, 36 hour and 48 hour cycles). The temperature cycled from 10°C to 20°C in all experiments. Heath's measurement of thermal tolerance was the "critical thermal maximum" which was defined as the point at which loss of coordination occurred in the fishes. The results showed that physiological adaptation is to the natural 24-hour thermoperiods. That such cycle responses are probably genetic and not derived from cycled conditioning during ontogeny was indicated by the non-cyclic nature of the temperature of the hatchery water from which Heath's fish were obtained. Fry et al. (1946) suggested that fish are better able to withstand temperature extremes when subjected to fluctuating rather than constant temperature acclimation.

Marked changes in electrolyte balance in the body fluids of fishes do occur when the temperature of the water is changed drastically over a period of minutes (Heinicke and Houston 1965; Houston 1962). Changes in electrolyte balance also occur when a temperature is raised or lowered and held at the new temperature (Hickman et al. 1964). It is nevertheless unlikely that a freshwater fish in its normal habitat would encounter such persistant changes or large temperature changes. In temperature records obtained from the Alberta Biological Station Reports made at Gorge Creek, a trout stream in the foothills of Alberta, there were no changes in water

temperature faster than 1°C per hour in the four years the temperature records have been kept. However, more gradual diurnal fluctuations in temperature, some as great as 8°-10°C, occur regularly during the hot summer months in this mountain stream as well as many other streams inhabited by fish. The increase in temperature in a stream such as Gorge Creek is partly due to the warming effect of the sun on the rocks and water, as well as the general increase in air temperature. The temperature in the creek starts rising at 7:00 A.M. as the sun is rising and increases at a rate of about 1°C per hour until 5:00 P.M. in the afternoon when the sun drops below the level of the mountains. The rate of increase of the rising phase of the temperature cycle is greatest when the sun is directly overhead, at twelve noon. On the falling phase of the temperature cycle, the temperature drop is most rapid after sundown after which time the temperature falls gradually to the minimum level by the following morning (Fig. 1). Temperature fluctuations appear to be largest during the month of August.

The rainbow trout (Salmo gairdneri) is quite common in Alberta streams although it is not a native fish to this province. Most mountain and foothill streams which do have rainbow trout have been stocked by the Fish and Wildlife Division of the Province of Alberta. The stocking of rainbow trout in Gorge Creek has been of limited success and it is thought that a contributing factor could be the large diurnal temperature fluctuations which occur in the late summer. However, hybrids of stocked rainbow trout and native cutthroat

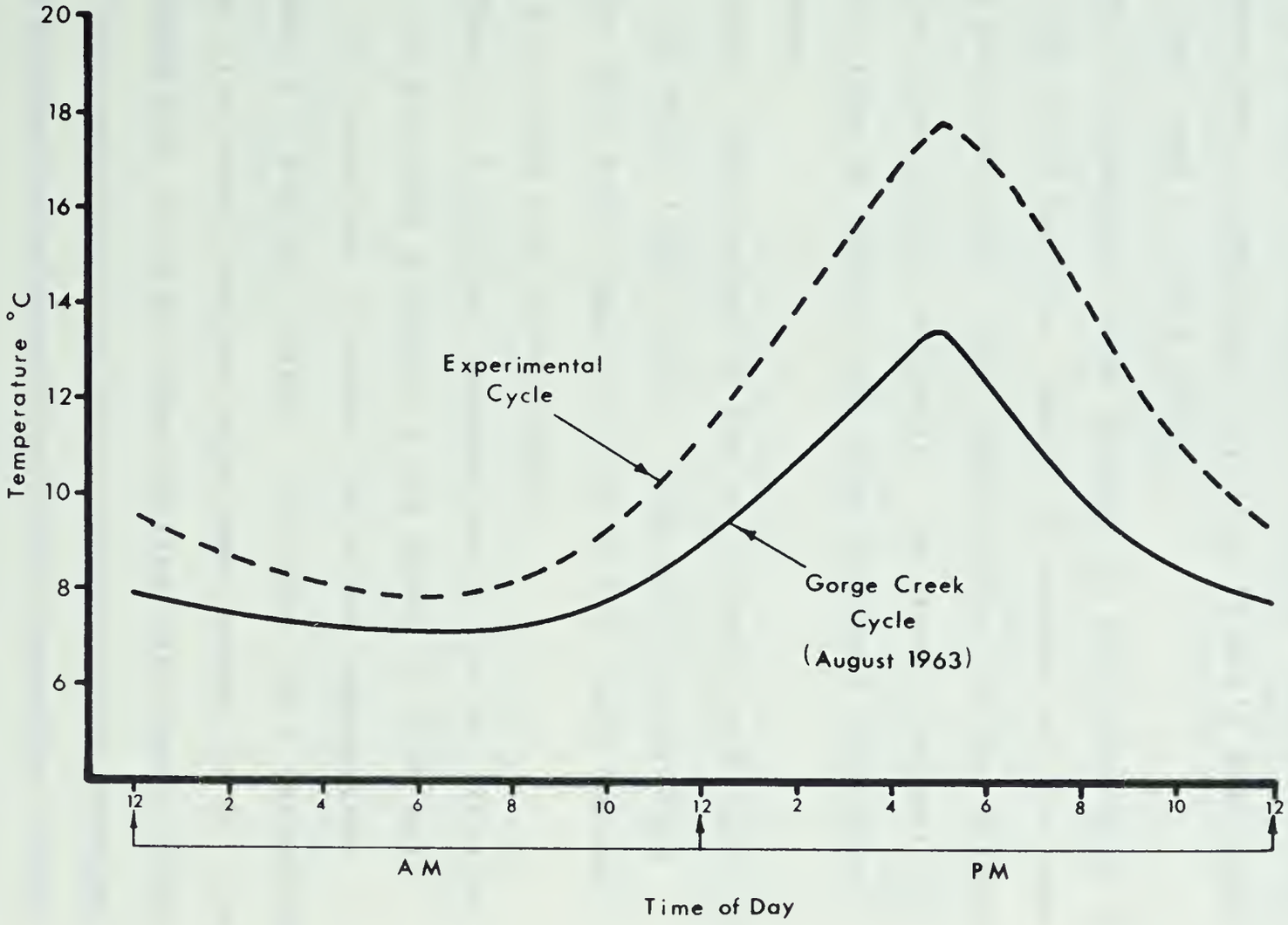
trout (Salmo clarkii) have successfully maintained a population in Gorge Creek.

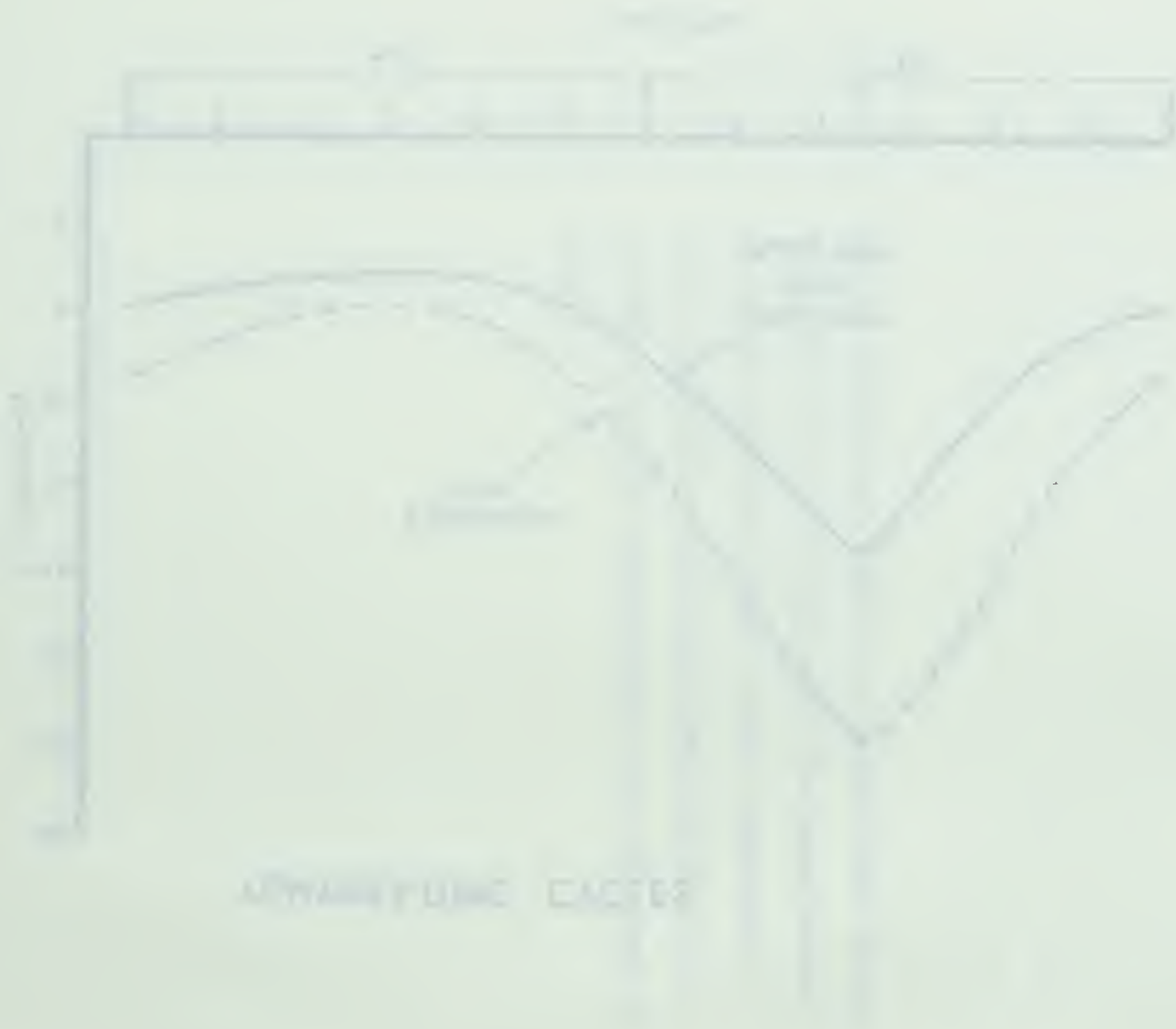
Because temperature conditions are so varied in mountain streams, the problem is presented as to how populations of fishes adjust or acclimate to these changes in the physical environment. Temperature compensation has been shown to be primarily a biochemical and cellular process, therefore changes in body fluid concentrations and electrolyte levels could serve to indicate how rainbow trout adjust to the temperature conditions in Gorge Creek.

My objectives were to find out if a cycling temperature has any effect on the body fluid and electrolyte composition in rainbow trout and to assess the possibility of acclimation to the cycling temperature at the ionic level.

Figure 1. Daily temperature cycles in Gorge Creek during the month of August, 1963 and the experimental cycle to which the trout in the laboratory were subjected. The Gorge Creek cycle is an average of daily temperatures for the month of August.

TEMPERATURE CYCLES





METHODS

Rainbow trout (Salmo gairdneri) used in this experiment were obtained from the Alberta Provincial Fish Hatchery in Calgary. The fish were maintained in the laboratory at the University of Alberta in Edmonton in running, dechlorinated water and were fed a commercial dry diet (Clark's Fish Food) similar to that used at the hatchery.

Fish were transported from Calgary to the University of Alberta in 180 litre barrels. About 10 percent of the trout died during the first four days after arrival at the University, but following this initial mortality, no trout died and all fish appeared free of fungus and other diseases throughout the experiment.

The natural photoperiod of the environment was simulated with a 12L-12D daily light cycle. Time-clock controlled lights in the fish tanks came on at 8:00 A.M. each morning and went off at 8:00 P.M. every night.

The effect of a cycling temperature on the electrolyte balance of the fish was studied in one group of fish held in a large 720 litre tank in which the water temperature cycled between 8°C and 18°C daily. To obtain this temperature cycle in the fish tank, heating units were turned on at 7:00 A.M. and off at 5:00 P.M. at which time cooling units were turned on to lower the temperature to 8°C by the following morning. Heating and cooling units were time-clock controlled. This daily cycle was designed to simulate the average daily temperature fluctuations of Gorge Creek in the month of August, 1963

(Fig. 1). The cycling fish (as they will be referred to) were acclimated to this cycling temperature for 42 days.

After the acclimation period the cycling fish were sampled at 8°, 10°, 12°, 14°, 16°, and 18°C on the rising phase of the temperature cycle and at 16°, 14°, 12° and 10°C on the falling phase of the cycle. Twelve fish were sampled at each of these temperature points. Because the temperature was always changing, only two fish were sampled at each temperature per day. Thus it took six days to sample 12 fish at each sample temperature. Sampling times and temperatures were carefully noted and it was found that times and temperatures for a group of fish sampled on one day at a particular temperature did not differ by more than fifteen minutes from fish sampled at ± 0.2°C of the same temperature on following days. In this way all fish sampled at a particular temperature had approximately the same amount of time to adjust to that part of the cycle.

Six groups of control fish were held in separate 150 litre tanks and acclimated for 42 days to water temperatures maintained at 8°, 10°, 14°, 16°, and 18°C respectively. The controlled photoperiod was 12 hours. Twelve fish were sampled at each temperature.

SAMPLING PROCEDURE

A 0.5 to 1.0 ml blood sample was collected in a heparinized syringe by tapping the dorsal aorta at a point dorsal to the anal fin of the fish. The sample was taken from unanaesthetized trout within 20 seconds of removal from the water.

This procedure was preferred to preliminary anaesthetization with MS222 to avoid possible physiological changes resulting from extreme hyperactivity which accompanied exposure to the anaesthesia. The blood sample was immediately centrifuged in a Beckman micro centrifuge and a small portion of the plasma was analyzed for water content with a Goldberg refractometer. This instrument measures the refractive index which is converted to percent plasma water. The remainder of the plasma sample was frozen and stored at -15°C for further chemical analysis. Any plasma sample which showed the slightest haemolysis was discarded.

Immediately after blood sampling, the fish was killed by a sharp blow on the head. A sample of the epaxial muscle was excised from the area below the dorsal fin, dissected free of skin and scales, put into a tared vial, and weighed. The sample was dried in a 105°C oven for sixteen hours, then cooled in a desiccator and weighed again to determine the water content of the tissue. The dried tissue was ground to a fine powder in a "Wig-l-bug" (Cresent Dental Mfg. Co. Chicago, Ill.) and stored at -15°C.

ANALYTICAL PROCEDURES

Plasma Analysis of Sodium and Potassium

Sodium and potassium concentrations in the plasma were determined with a Perkin-Elmer flame photometer, Model 146, using a low temperature propane-air mixture flame. A cool flame such as the flame given by a propane-air mixture is preferable because it avoids ionization interference such

as the enhancement of the sodium emission by the presence of potassium. Tests showed that the flame photometer readings of plasma sodium or plasma potassium were unaffected by the presence of the opposite ion. Plasma samples were diluted 100 fold with distilled, demineralized water. Standard sodium and potassium solutions of desired concentrations were made by adding weighed amounts of reagent grade NaCl and KCl to distilled, demineralized water.

Tissue Sodium and Potassium

A sample of finely ground epaxial muscle weighing approximately 0.1 gram was placed in a pre-weighed ignition tube. This was dried for 16 hours at 105°C, cooled in a desiccator, and weighed to 0.01 percent accuracy. The sample was incinerated in a muffle furnace for five hours at 550°C. The ash was diluted with 5 ml of distilled water and analyzed for sodium and potassium content with the Perkin-Elmer flame photometer. It was found that the high concentration of potassium in the tissue enhanced the sodium flame emission by as much as 15 percent. However sodium was found not to enhance or decrease the potassium flame emission. It was therefore possible to run a series of correction curves to correct for potassium background on the sodium signal (Appendix III). The sodium concentrations of all tissue samples were corrected for potassium interference determined from the actual amount of potassium in the sample.

Tissue Chloride

Tissue chloride was measured by the method of alkaline digestion of chloride described by Cotlove (1963b). Approximately 0.07 grams of the dried, ground tissue was dried 16 hours at 105°C in a 12 ml pre-weighed test tube. After cooling in a dessicator the sample was accurately weighed to determine the exact amount of tissue being analysed. Three ml of 0.6 N NaOH were added to the sample, the tube was capped with aluminum foil and placed in a boiling water bath for 30 minutes. Following total tissue dissolution with the alkali, the protein was precipitated with 3 ml of 4% $ZnSO_4$ in 0.4 N HNO_3. The suspended precipitate was stirred periodically for one hour on a Vortex Junior Mixer and then centrifuged for 20 minutes at 5900 g and 15°C in a Sorvall RC-2 refrigerated centrifuge. Duplicate 2 ml samples of the supernatant were pipetted into 6 ml chloride vials. To oxidize interfering sulfhydryl groups released by the alkaline digestion of the tissue (Cotlove 1963b), 0.1 ml of perborate solution (0.2 M $NaBO_3$ in 2.8 N NaOH) was added to each sample vial. After standing for 24 hours, 50 µl of gelatin indicator solution (Buchler Instruments, Inc.) and 0.5 ml of 1.3 N HNO_3 in 50% acetic acid were added to the sample.

Standard solutions were prepared by following the same procedure except that the plasma sample was omitted. Prior to the addition of the perborate solution in a standard vial, 100 µl of 10.4 mEq/L chloride solution was added. Method blanks were also prepared by duplicating all steps in the

chloride extraction technique, without the addition of sample or standard. Samples, standards and blanks were then measured for chloride content by automatic silver-ion titration with the Buchler-Cotlove chloridometer (American Instrument Co., Silver Springs, Maryland). The validation of the alkaline digestion of chloride from biological tissues has been demonstrated by Cotlove (1963a) using the chlorine-36 isotope dilution method.

Plasma Chloride

Chloride in plasma was determined on 50 µl samples combined with 4 ml of nitric acetic reagent (0.1 N HNO_3 and 10% glacial acetic acid) in chloride vials. Standards were also prepared by adding 50 µl of 104 mEq/L chloride solution to 4 ml of the nitric acetic reagent. Method blanks of 4 ml of the nitric acetic reagent were prepared. After the addition of 100 µl of gelatin indicator solution, samples, standards, and blanks were run on the Buchler-Cotlove chloridometer.

In all chloride determinations, Lab-trol (Dade Medical Supplies) was used as a standard solution. Lab-trol is a control serum in liquid form containing known concentrations of substances found in blood.

Protein Binding of Chloride in the Plasma

The nitric acetic reagent (Cotlove 1963b) used in the analysis of plasma chloride does not digest the protein in the plasma and it is possible that any chloride which is bound to the plasma proteins would not be measured using the potentiometric silver ion titration technique. If there is

any protein binding, the question arose as to whether the binding was temperature dependent. A simple experiment was designed to attempt to answer both questions.

Ten trout maintained at 4°C were blood sampled at the Calgary Fish Hatchery and plasma frozen immediately for further analysis. An additional 10 trout, naturally acclimated at the Raven Fish Rearing Station at Raven, Alberta at a water temperature of between 12 and 15°C, were also sampled.

100 µl of plasma from each fish was analyzed for plasma chloride using the method already described. An additional 100 µl sample of plasma from each trout was digested in warm 0.6 N NaOH to break down the plasma proteins and release any protein-bound chloride that might be present. The remaining peptide fragments were precipitated with 4% $ZnSO_4$ in 0.4 N HNO_3 and 2 ml samples of the supernatant were put into chloride vials. Oxidation of sulfhydryl groups, which interfere with titration, was accomplished by sodium perborate treatment as described in the previous section on Tissue Chloride Analysis.

After the addition of the gelatin indicator solution, all samples were run on the Buchler-Cotlove Chloridometer. Standard solutions for all tests were prepared in the same manner as the test samples, differing only in that a known amount of chloride solution was added to each vial in place of the sample.

Statistical Analysis of the Results

The results of this study were programmed for analysis of variance at the University of Alberta Computer Center.

The values of F obtained from the analyses and their levels of significance are shown for the cycling fish as well as the control fish, in the figure legend of each graph (Figs. 2-14). The "Students t-Test" was calculated between cycling fish and control fish at the same temperature to determine levels of significant differences between the two.

Group means in the cycling fish were compared for levels of significant differences by means of the Duncan's multiple range test (Duncan 1955). Comparisons between the means of the cycling fish are given by the range test on each graph (Figs. 2-14). For example, in the graph of muscle sodium (Fig. 6) the lines underscore groups (or sub-sets) of means within which it is not possible to demonstrate significant differences. Duncan defines this series of comparisons very well:- "Each difference is significant if it exceeds the corresponding shortest significant range; otherwise, it is not significant .... The sole exception to this rule is that no difference between two means can be declared significant if the two means concerned are both contained in a sub-set of the means which has a non-significant range." In Fig. 6, means 5, 2, and 3 are not significantly different. Means 8, 6, and 4 as well, are not significantly different but 5, 2, and 3 are significantly different from 6, 4, 9, 10, 7, and 1 because no single line underscores all of them. Similarly in the graph of muscle water (Fig. 3), mean number 1 is significantly different from all other means, and all the other means do not differ significantly from each other.

Formulae used to calculate sodium space, chloride space,

and chloride-potassium space are given in Appendix I. Individual data for each fish are given in Appendix II.

RESULTS

MUSCLE WATER

Plasma water and muscle water measurements from trout subjected to a cycling temperature and from controls are shown in Figs. 2 and 3. Totalmuscle water in the cycling fish ranged from 78.30% to 79.61% while that of the control fish ranged from 76.80% to 79.68%. These values are well within the normal range for rainbow trout given by Hickman et al. (1964) and Schiffman and Fromm (1959). Muscle water showed a small but significant increase in the cycling fish when the temperature rose from 8°C to 10°C (Fig. 3). There was no further change throughout the remainder of the temperature cycle. In contrast, the control fish at temperatures above 14°C show a marked drop in muscle water. It appears that in the cycling fish, the short period of exposure to temperatures above 14°C (approximately 6 hours) was too brief to affect the muscle water content. In the cycling fish the low level of muscle water at 8°C could reflect the relatively longer period of exposure (12 hours overnight) to the lower temperatures of the cycle.

The plasma water content in the cycling fish did not differ significantly from one temperature to another in the cycle. Plasma water in the control fish did not differ significantly from one holding temperature to the other although the levels for the control fish were 0.25 to 2.0% lower than the cycling fish. Differences at the 5% level of significance between the controls and the cycling fish only occurred at 18°C and at 16 and 10°C on the falling phase of

the cycle.

The coefficient of condition, an expression of "plumpness" of a fish, was determined on all fish to indicate either a significant loss or gain of body water following a temperature change. The coefficient of condition is an empirical relation between body weight and body length*. A significant rise in the coefficient within a period of hours in a fasting fish would indicate an increase in body weight, presumably by the gain of water from the environment.

In the control fish the increase in coefficient from 1.04 at 14°C to 1.22 at 18°C represents an average 12.4% increase in body weight (Fig. 4). In the cycling fish, the variations in coefficient are not significant. Significant differences in the coefficient do however occur between the cycling and control fish at the same temperature: at 12° and 16°C on the rising phase of the cycle, at 18°C, and at 16°, 12° and 10°C on the falling phase of the cycle. At 18°C the difference in coefficient represents a difference of 20% in body weight. Changes in the coefficient in the control fish, such as the observed increase at higher temperatures, indicate faster growth at the higher temperature, rather than a shift in body water in direct response to temperature change.

* Coefficient of Condition = $\frac{100 \times \text{body weight in grams}}{(\text{fork length in cm.})^3}$

Figure 2 Variations which occur in plasma water between the cycling fish (solid line) and the control fish (dashed line). Mean values for the cycling fish are shown as closed circles. Mean values for the control fish are shown as open circles. The vertical line through each mean represents ± one standard error of the mean. Means of the cycling fish are numbered for comparison to one another by Duncan's multiple range test shown on the right hand side of each graph. Values of *F* obtained by analysis of variance and their levels of significance are shown at the bottom of the figure legend of each graph. Significant differences at the 5% level between the cycling and control fish at one temperature are indicated by a star above that temperature on the abcissa of the graph.

F (cycling)=1.30 (N.S.)

F (control)=0.87 (N.S.)

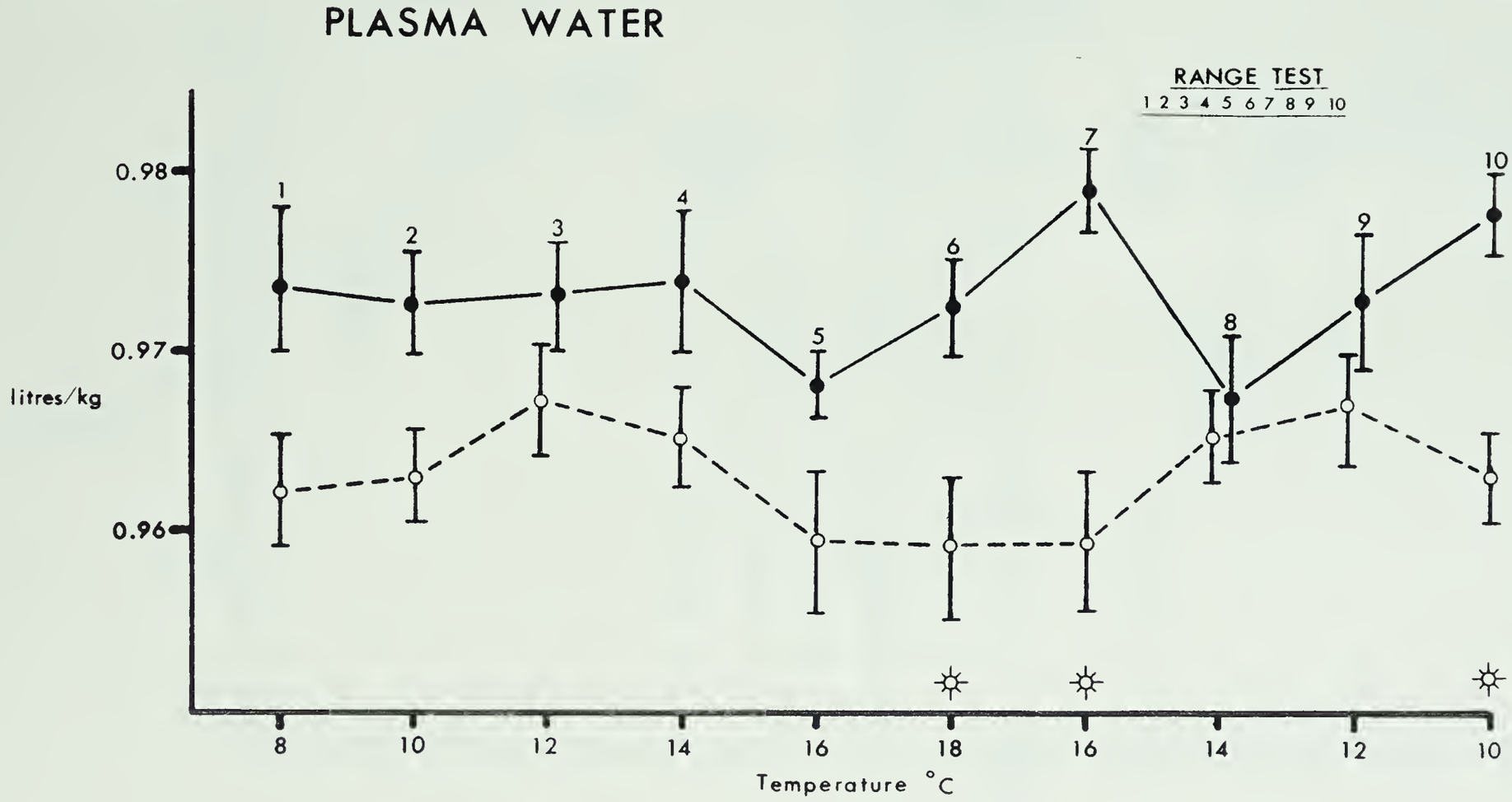

PLASMA WATER
RANGE TEST
1 2 3 4 5 6 7 8 9 10
0.98
0.97
0.96
litres/kg
1
2
3
4
5
6
7
8
9
10
8
10
12
14
16
18
16
14
12
10
Temperature °C

Figure 3 Variations in muscle water in the cycling fish (solid line) and in the control fish (dashed line). Graphical presentation is the same as in Fig. 2.

F (cycling) $= 3.01 > 1.98_{.05}$

F (control) $= 15.97 > 2.38_{.05}$

MUSCLE WATER

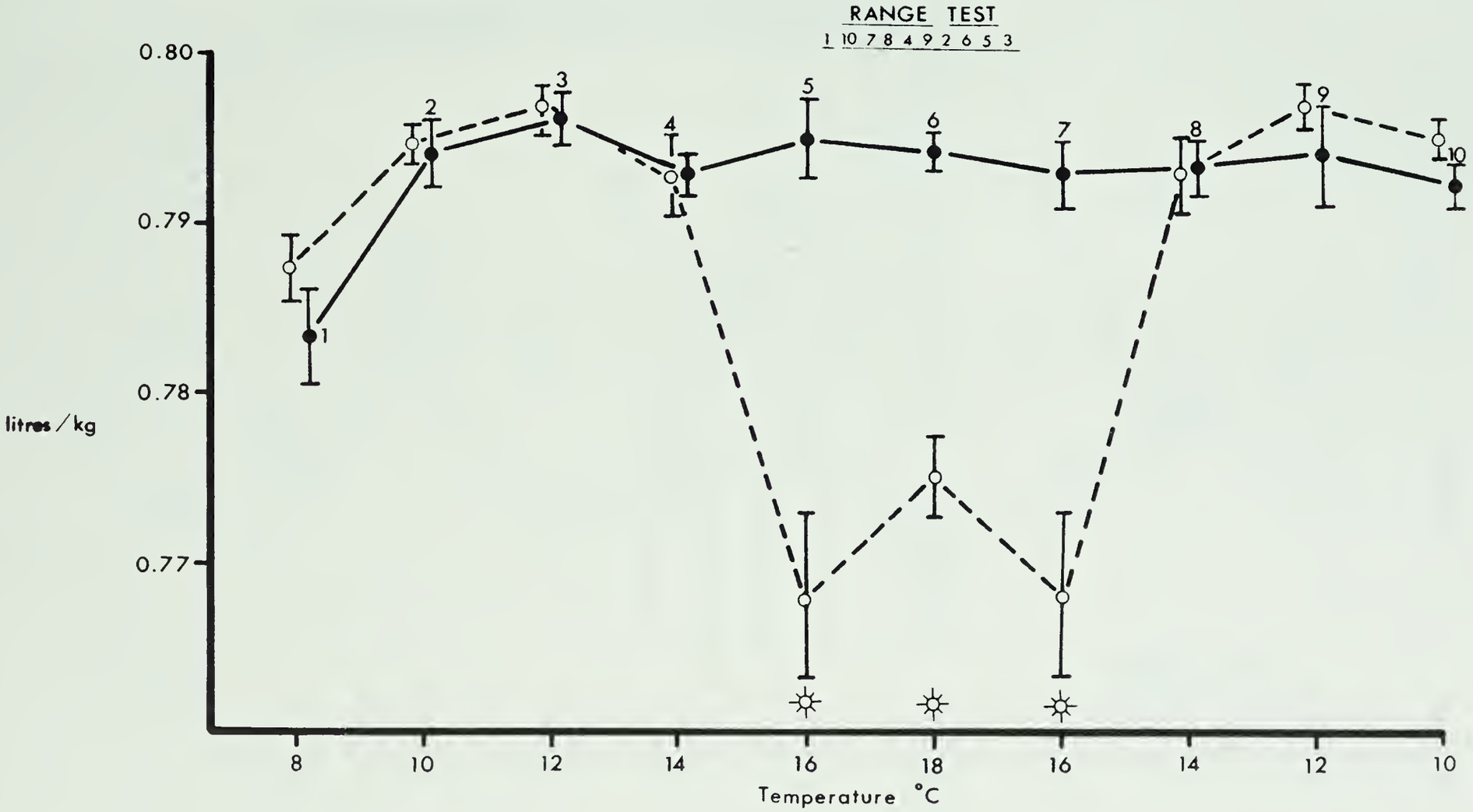
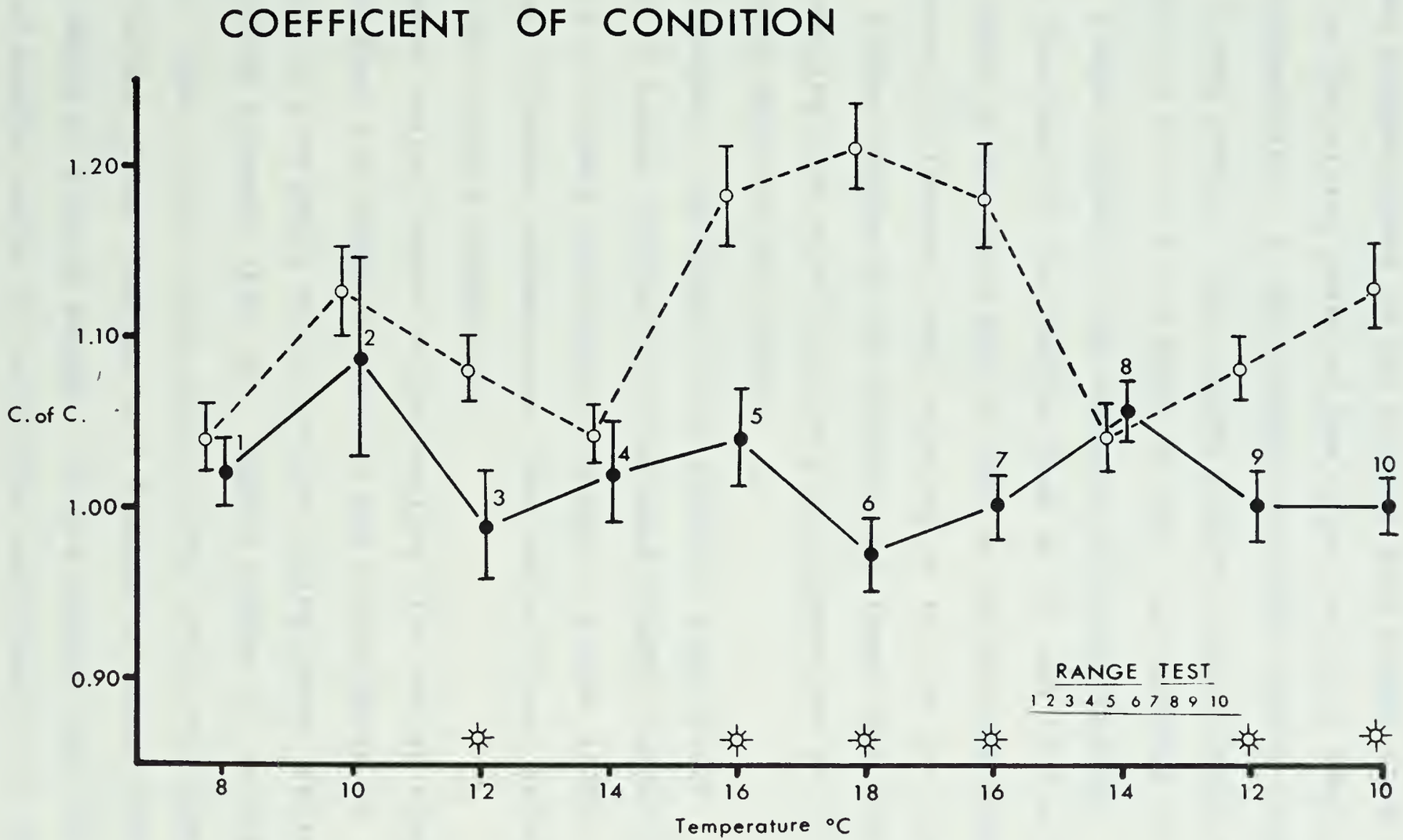


Figure 4 Differences in coefficient of condition in the cycling fish (solid line) and in the control fish (dashed line). Graphical presentation is the same as in Fig. 2.

F (cycling) = 1.68 (N.S.)

F (control) = $9.25 > 2.38_{.05}$


COEFFICIENT OF CONDITION
1.20
1.10
1.00
0.90
C. of C.
1
2
3
4
5
6
7
8
9
10
RANGE TEST
1 2 3 4 5 6 7 8 9 10
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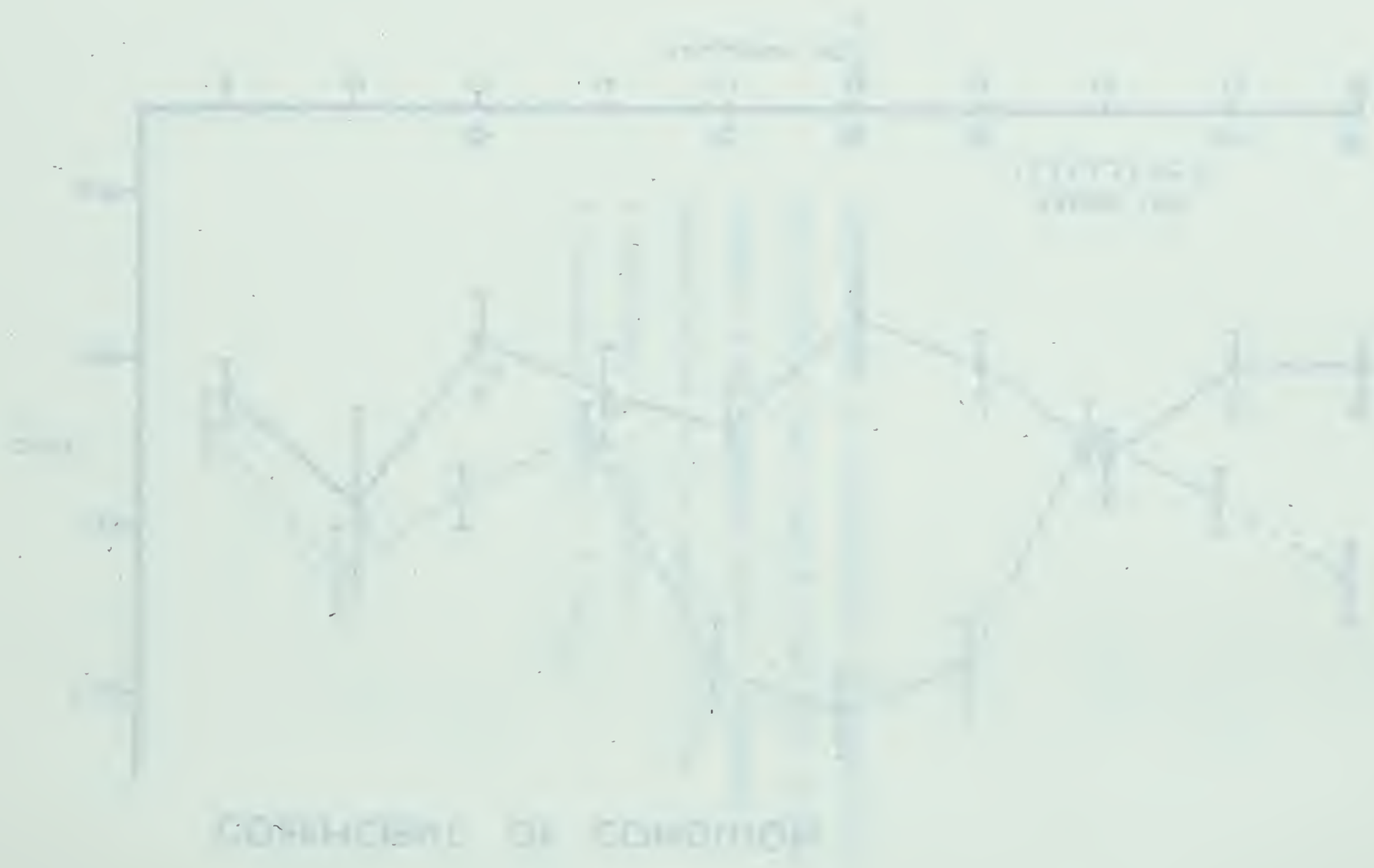
10
Temperature °C



MUSCLE AND PLASMA SODIUM

Mean sodium values in the cycling fish ranged from 90 mEq/L at 10°C on the rising phase of the cycle, to 118.5 mEq/L at 18°C, representing a 23.8% increase in plasma sodium concentration (Fig.5). In the control fish, plasma sodium values ranged from 93.3 mEq/L at 8°C to 137.4 mEq/L at 18°C, an increase of 32.2%. Plasma sodium of the controls was significantly greater than that of the cycling fish at 14° and 16°C on the rising phase of the cycle and at 14°C on the falling phase. The greatest difference, 28%, occurred at 16°C on the rising phase. Plasma sodium in the controls was lower than that of the cycling fish at 8°C on the rising phase and at 10°C on the falling phase of the cycle.

In summary, the control fish showed a significant increase in plasma sodium with increased holding temperatures. Cycling fish showed significant changes in plasma sodium levels at different temperatures, but not in direct relationship to the cycling temperature.

Muscle sodium levels in the cycling fish varied significantly from 3.05 mEq/L at 16°C on the rising phase of the cycle to 6.99 mEq/L at 8°C on the rising phase, reflecting a 112.8% increase (Fig. 6). Muscle sodium is, on the average, lower on the rising phase of the cycle and higher on the falling phase (average of 3.8 mEq/L on rising phase and 5.7 mEq/L on falling phase). In the control fish the levels of muscle sodium do not differ significantly at different temperatures. Muscle sodium is significantly lower in the cycling fish at 10°, 12°, and 16°C on the rising phase of

Figure 5 Plasma sodium level differences in the cycling fish (solid line) and in the control fish (dashed line). Graphical presentation is the same as in Fig. 2.

$F_{(cycling)} = 1.99 > 1.98_{.05}$

$F_{(control)} = 10.53 > 2.38_{.05}$

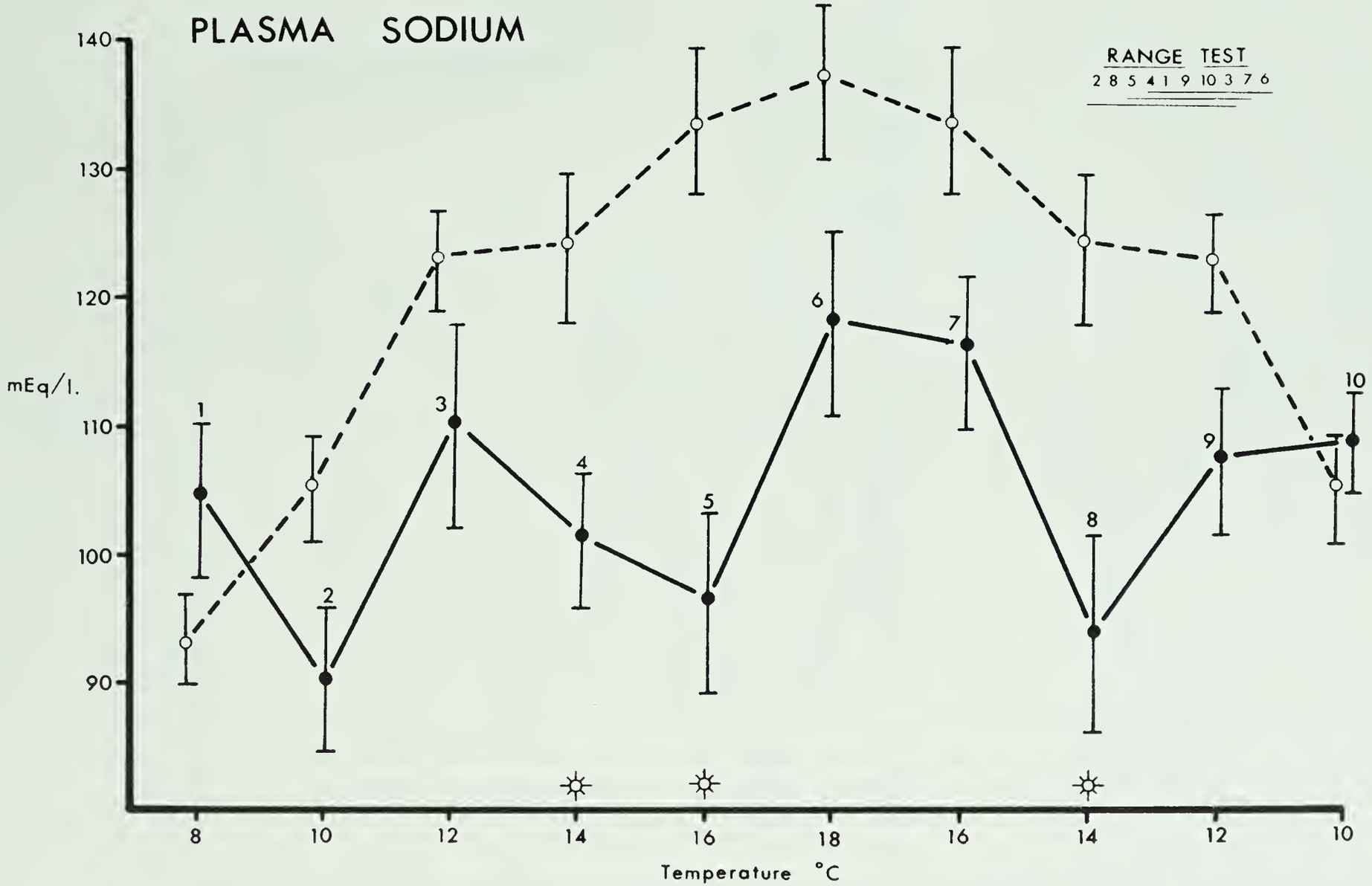
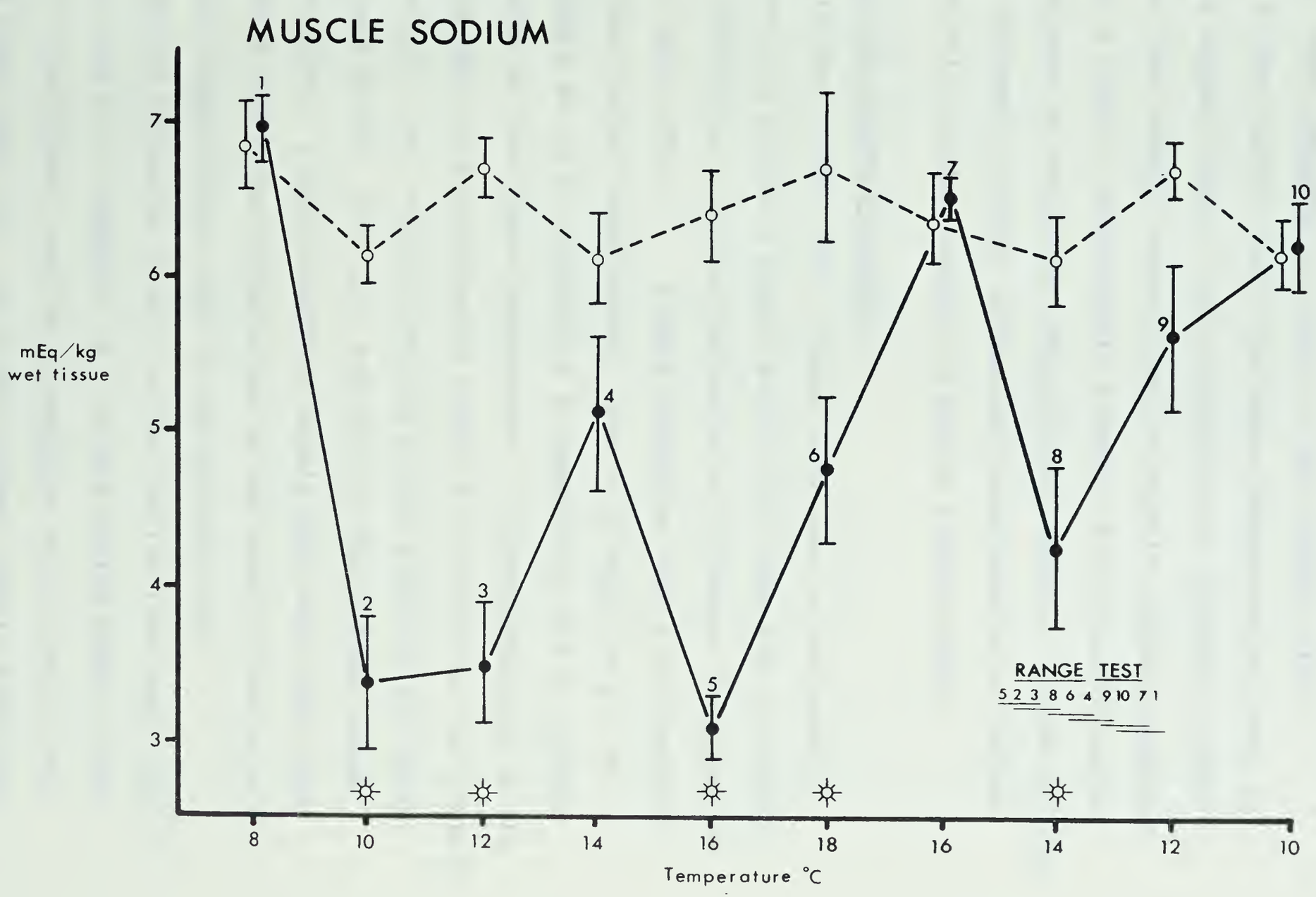

PLASMA SODIUM
RANGE TEST
2 8 5 4 1 9 10 3 7 6
140
130
120
110
100
90
mEq/l.
1
2
3
4
5
6
7
8
9
10
8
10
12
14
16
18
16
14
12
10
Temperature °C

Figure 6 Differences in muscle sodium levels in the cycling fish (solid line) and in the control fish (dashed line). Graphic presentation is the same as in Fig. 2.

F (cycling) = $12.82 > 1.98_{.05}$

F (control) = 0.949 (N.S.)


MUSCLE SODIUM
mEq/kg
wet tissue
7
6
5
4
3
1
2
3
4
5
6
7
8
9
10
RANGE TEST
5 2 3 8 6 4 9 10 7 1
8
10
12
14
16
18
16
14
12
10
Temperature °C

the cycle, at 18°C and at 14°C on the falling phase of the cycle, when compared to levels in the control fish.

In the control fish, the large changes which occur in plasma sodium levels, with acclimation to different temperatures, are totally absent from the muscle sodium concentrations, a fact of considerable interest since muscle sodium is believed to be mostly extracellular sodium. In the cycling fish, changes in plasma sodium are associated to a limited degree with similar changes in muscle sodium levels.

MUSCLE AND PLASMA POTASSIUM

In the cycling fish, plasma potassium varied greatly between fish within a sample period and there was no significant difference in plasma potassium at different temperatures of the cycle (Fig. 7). Plasma potassium levels in the control fish varied significantly from 6.52 mEq/L at 8°C to 4.10 mEq/L at 18°C, reflecting a decrease of 37% in plasma potassium at the higher temperature. Plasma potassium in the cycling fish is significantly higher than levels in the control fish at 12°, 14°, and 16°C on the rising phase of the cycle, 18°C, and at 16°, 14°, 12°, and 10°C on the falling phase of the cycle. Plasma potassium levels were much less variable at a particular temperature in the control fish held at constant temperatures, than in the cycling fish.

Muscle potassium levels in the cycling fish vary significantly from 90.79 mEq/kg at 12°C on the rising phase of the cycle; 55.8% higher than 58.30 mEq/kg at 16°C, also on the falling phase (Fig. 8). As in the plasma, muscle potassium

Figure 7 Changes in plasma potassium levels in the cycling fish (solid line) and in the control fish (dashed line). Graphic presentation is the same as in Fig. 2.

F (cycling) = 1.92 (N.S.)

F (control) = $53.64 > 2.38_{.05}$

PLASMA POTASSIUM

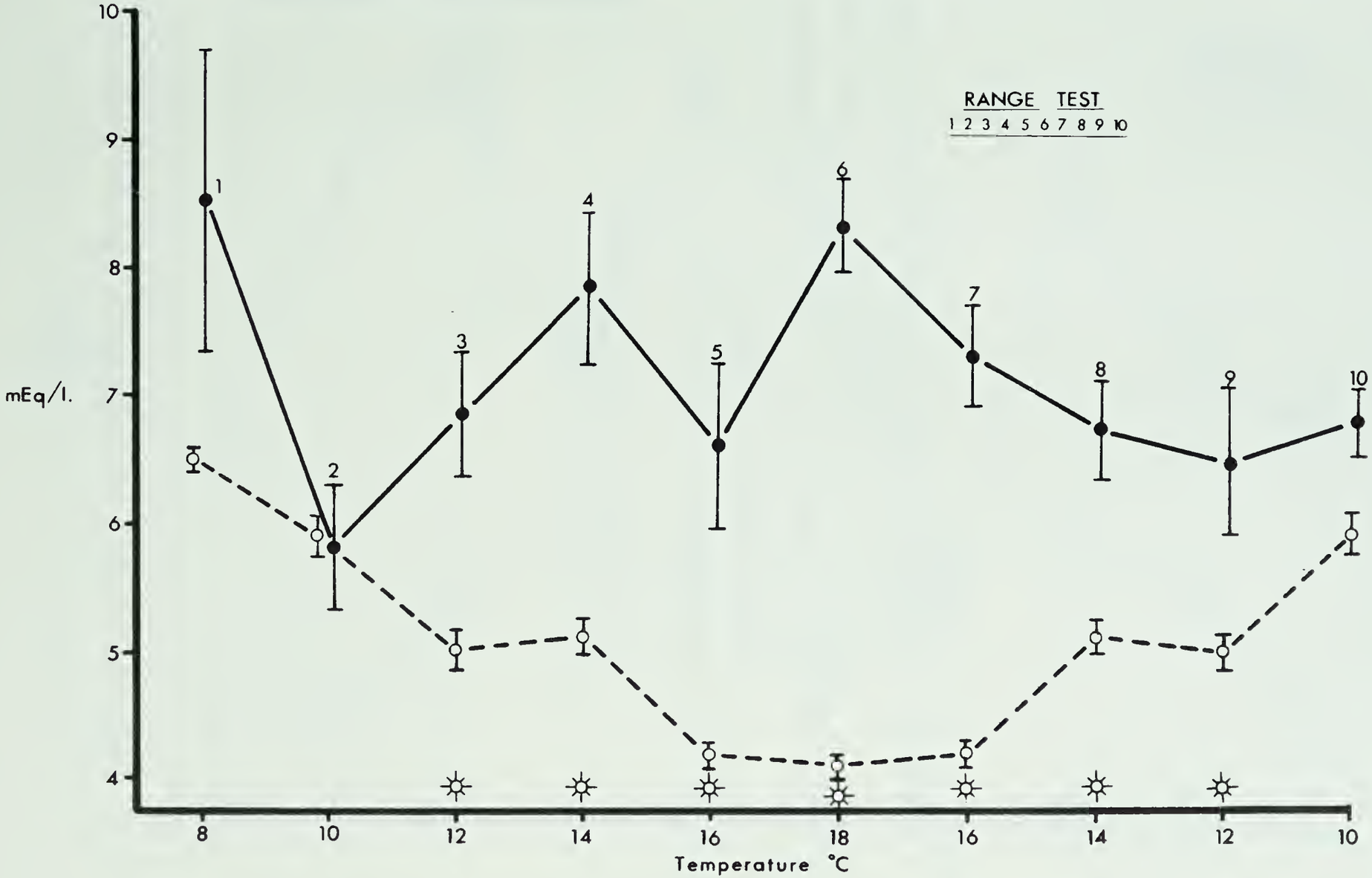
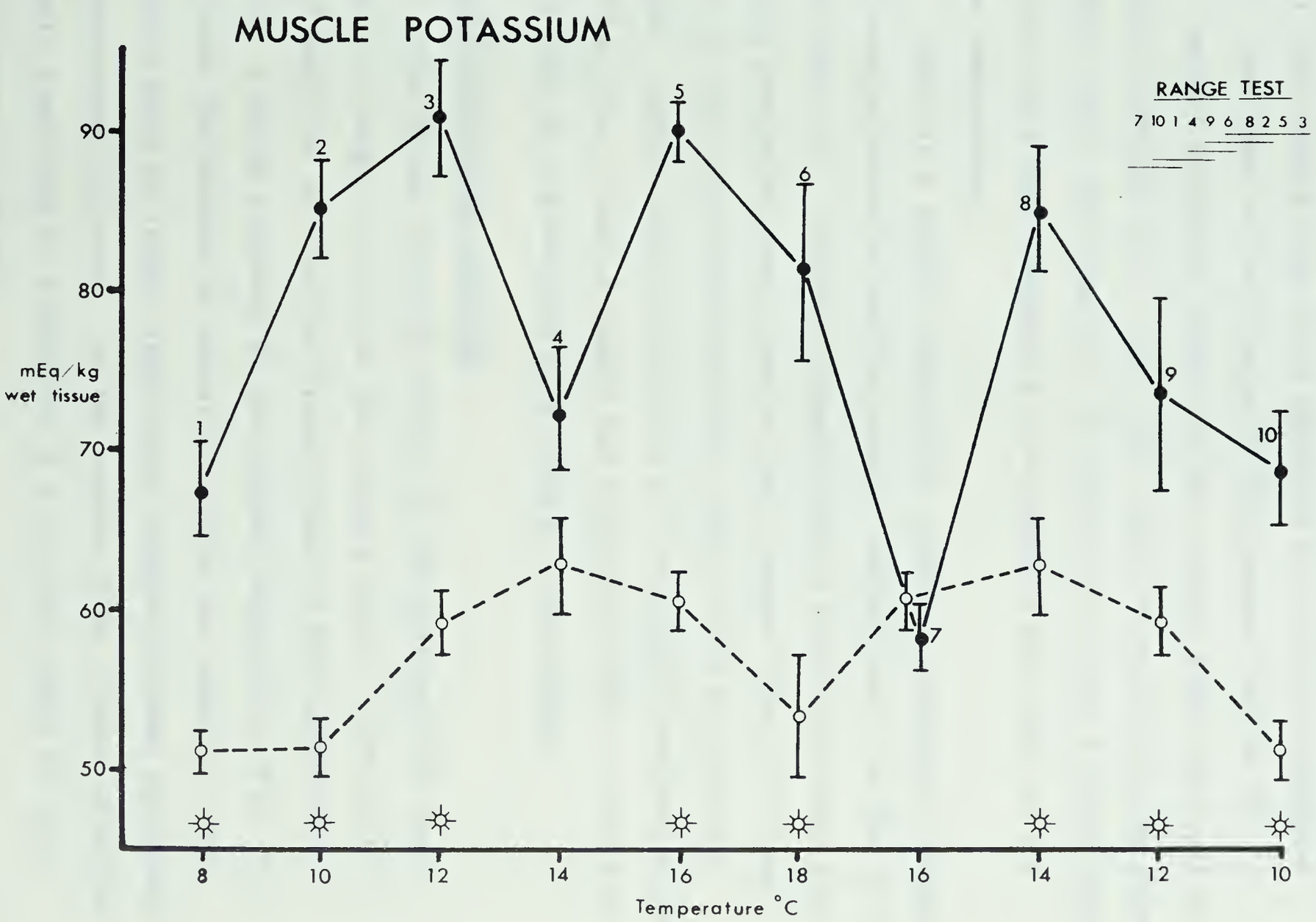
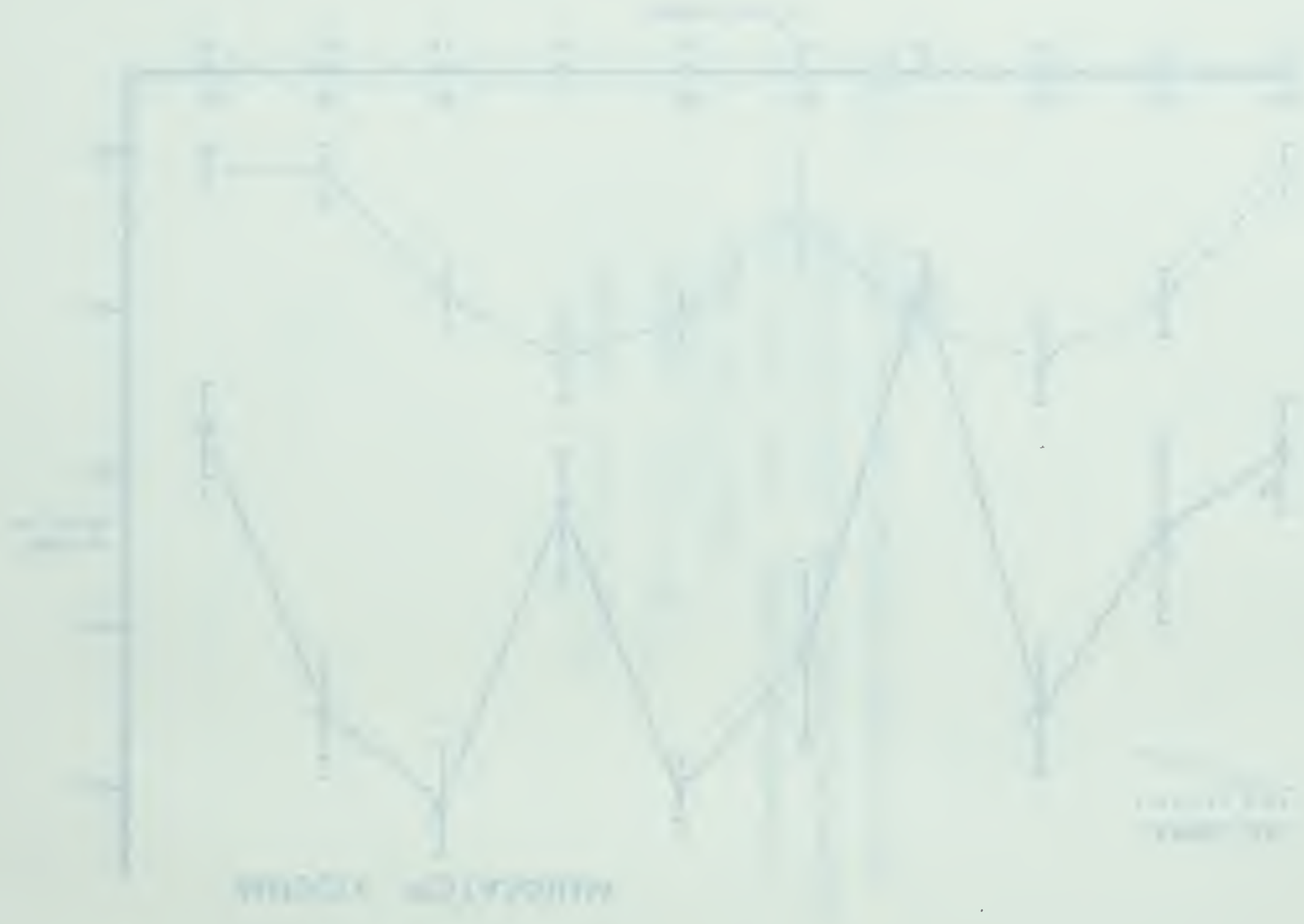


Figure 8 Changes in muscle potassium levels in the cycling fish (solid line) and in the control fish (dashed line). Graphic presentation is the same as in Fig. 2.

F (cycling) $= 8.07 > 1.98_{.05}$

F (control) $= 4.31 > 2.38_{.05}$


MUSCLE POTASSIUM
RANGE TEST
7 10 1 4 9 6 8 2 5 3
90
80
70
60
50
mEq/kg
wet tissue
1
2
3
4
5
6
7
8
9
10
8
10
12
14
16
18
16
14
12
10
Temperature °C



varied much less within each sample temperature in the control fish than in the cycling fish. Muscle potassium in the control fish varied from 51.24 mEq/kg at 8°C to 63.10 mEq/kg at 14°C, the level at 14°C being 21.6% greater than at 8°C.

Muscle potassium levels in the cycling fish are significantly different at 8°, 10°, 12°, and 16°C on the rising phase of the cycle, 18°C and 14°, 12°, and 10°C on the falling phase of the cycle when compared to the control fish held at the same temperature.

There is a highly significant inverse correlation between the muscle potassium and the muscle sodium levels in the cycling fish (Fig. 9). At those temperatures of the cycle where the muscle potassium levels are at their lowest levels (8°C and 14°C on the rising phase and 16°C and 10°C on the falling phase), muscle sodium levels are at the highest concentrations of the cycle. Muscle sodium and potassium levels in the control fish do not exhibit this inverse relationship.

MUSCLE AND PLASMA CHLORIDE

Mean plasma chloride levels in the cycling fish vary from 51.0 mEq/L at 12°C on the falling phase of the cycle to 85.8 mEq/L at 14°C on the rising phase of the temperature cycle, a 68.4% increase at the higher temperature (Fig. 10). Although the degree of variation within one sample in the cycling fish is large, significant changes between different temperatures do occur. In the control fish, plasma chloride levels rose from 67.5 mEq/L at 8°C to 108.7 mEq/L at 12°C, an increase of 38.4%.

Figure 9 Comparison between the muscle sodium levels in the cycling fish (solid line) and the muscle potassium levels of the cycling fish (dashed line) to show the inverse relationship between the two. This graph is a composite of Figs. 6 and 8.

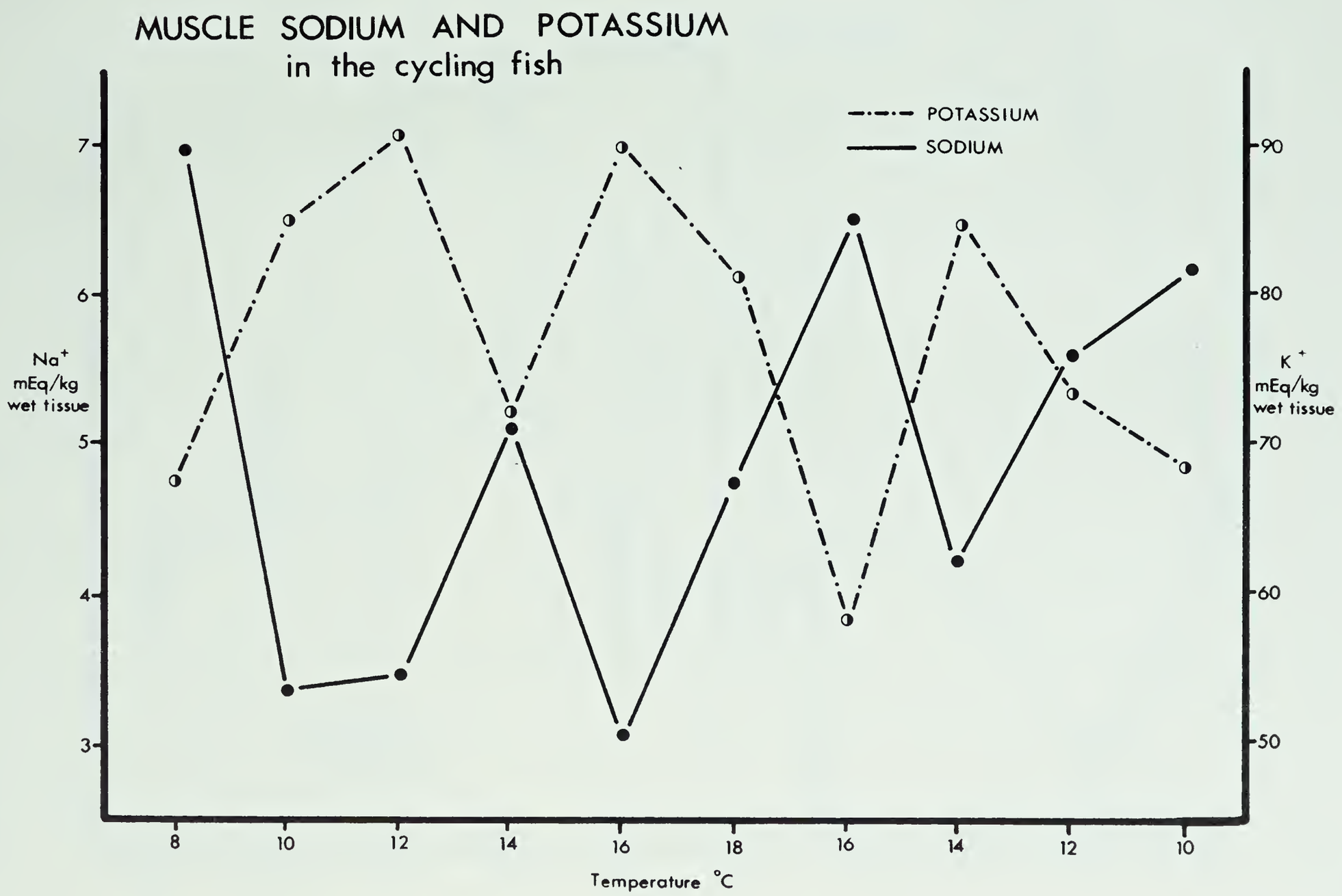
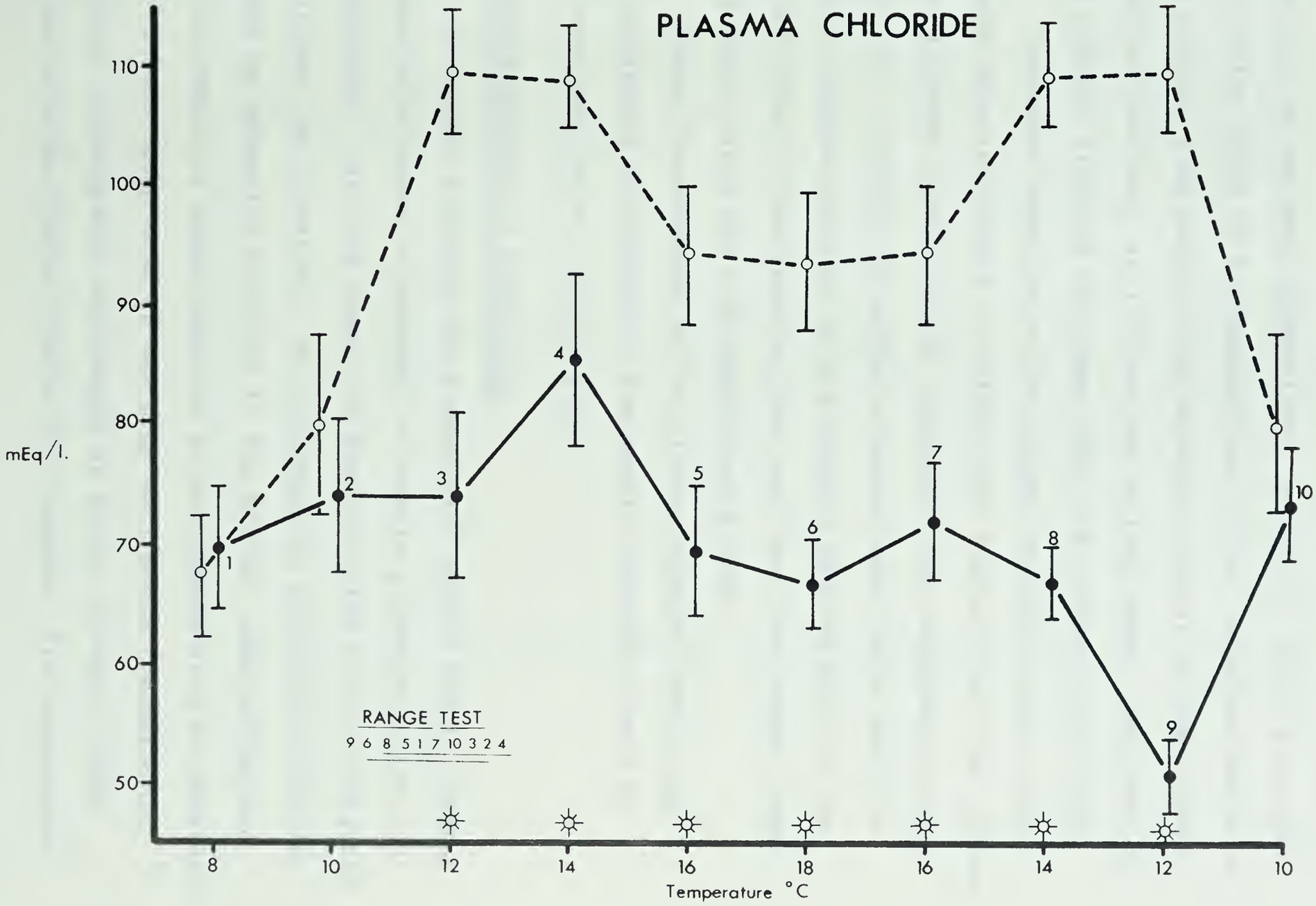

MUSCLE SODIUM AND POTASSIUM
in the cycling fish
POTASSIUM
SODIUM
Na+ mEq/kg wet tissue
K+ mEq/kg wet tissue
7
6
5
4
3
90
80
70
60
50
8
10
12
14
16
18
16
14
12
10
Temperature °C

Figure 10 Differences in plasma chloride in the cycling fish (solid line) and in the control fish (dashed line). Graphic presentation is the same as in Fig. 2.

F (cycling) $= 2.56 > 1.98_{.05}$

F (control) $= 7.68 > 2.38_{.05}$


PLASMA CHLORIDE
mEq/l.
110
100
90
80
70
60
50
1
2
3
4
5
6
7
8
9
10
RANGE TEST
9 6 8 5 1 7 10 3 2 4
8
10
12
14
16
18
16
14
12
10
Temperature °C

With the exception of 8°C and 10°C, all control fish had significantly higher plasma chloride levels than the cycling fish did at the same temperature. From 12°C to 18°C on both the rising phase of the temperature cycle, chloride levels in the cycling fish were 20-30% below the levels of the control fish; in one case, at 12°C on the falling phase, the level of the cycling fish was 53% lower than the control fish level.

In marked contrast to the plasma chloride levels, the muscle chloride levels in neither the controls nor the cycling fish differed significantly throughout the temperature cycle (Fig. 11). Variations within a sample were quite small in muscle chloride samples of the cycling fish as well as the control fish, illustrated by the fact that the largest standard error value was 0.50 mEq/kg wet tissue.

Large fluctuations in the plasma chloride levels are not reflected in changes in the muscle chloride levels of the cycling fish or control fish.

CHLORIDE BINDING IN THE PLASMA

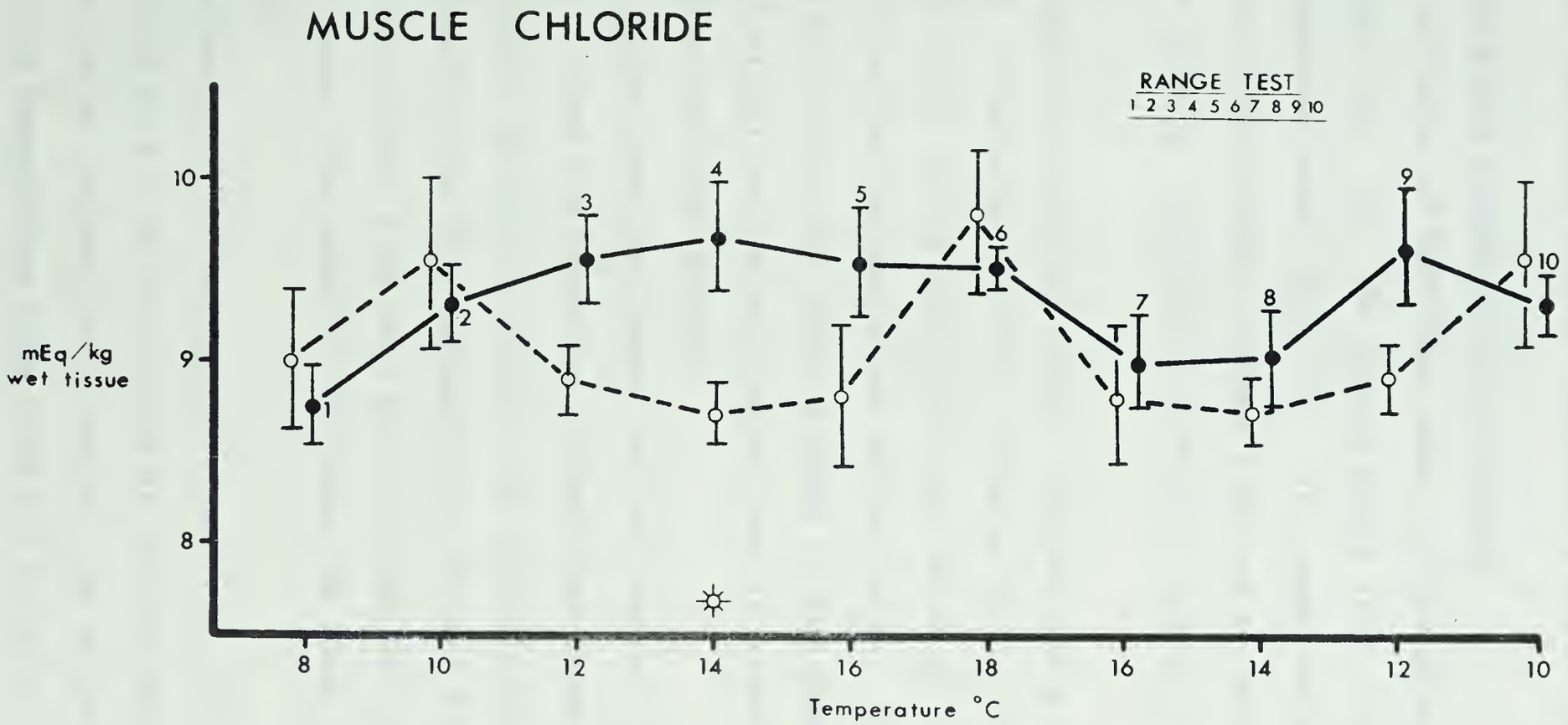
By simply diluting the plasma with nitric acetic reagent (description given in Methods) to obtain plasma chloride concentrations, only the levels of free chloride will be measured by silver ion titration. By utilizing an alkaline extraction method to determine chloride in the plasma, some estimation of the amount of bound chloride in the plasma will be obtained.

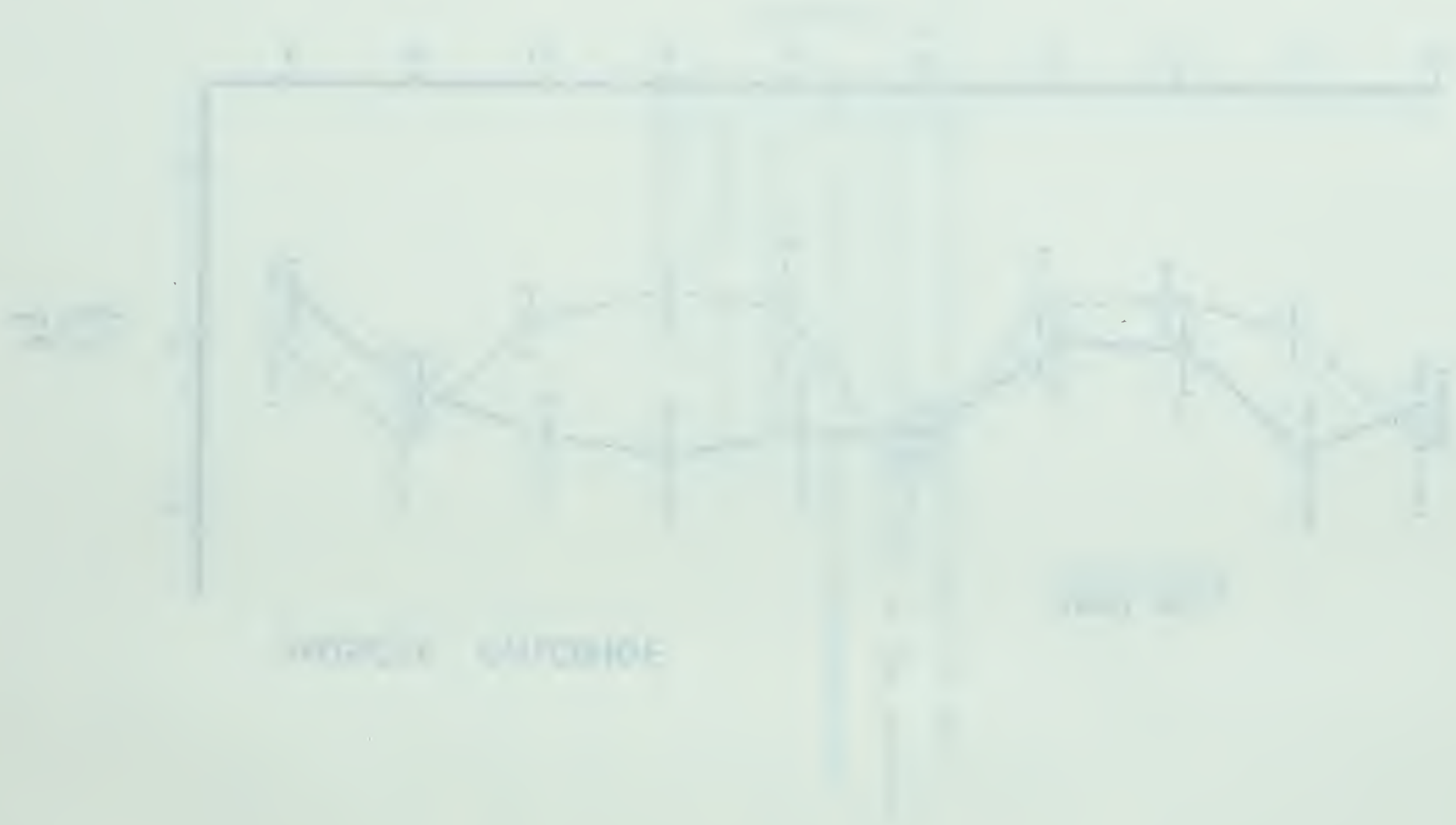
It was found that at the high acclimation temperature of 12-15°C (naturally acclimated at Raven, Alberta) there was no chloride binding (Table I). However, fish maintained

Figure 11 Changes in muscle chloride levels in the cycling fish (solid line) and in the control fish (dashed line). Graphic presentation is the same as in Fig. 2.

F (cycling) = 1.53 (N.S.)

F (control) = 1.55 (N.S.)


MUSCLE CHLORIDE
RANGE TEST
1 2 3 4 5 6 7 8 9 10
mEq/kg
wet tissue
10
9
8
1
2
3
4
5
6
7
8
9
10
8
10
12
14
16
18
16
14
12
10
Temperature °C



at the Calgary Fish Hatchery at 4°C show, on the average, that 15.8% of the total plasma chloride is bound (Table I).

EXTRACELLULAR FLUID VOLUME (ECFV) OF MUSCLE

Three estimates of ECFV were made in this study, the chloride space (Fig. 12), the sodium space (Fig. 13), and the chloride-potassium space (Fig. 14). All space estimates were calculated using the Donnan factors supplied by Manery (1954). The formulae used to calculate the various spaces are in Appendix 1.

Much variation occurred in the chloride space in the cycling fish. The value of 126.5 ml/kg at 14°C on the rising phase of the cycle is 37% lower than the value of 200.3 ml/kg found at 12°C on the falling phase of the cycle. The chloride space value at 12°C on the falling phase of the temperature cycle (200.3 ml/kg) is the only point that is significantly different from the other points.

The chloride space estimates for the control fish are interesting in that the estimates, other than those at 8°C or 10°C of either end of the cycle, are significantly lower than the estimates for the corresponding cycling fish. In most cases the control fish were 25-40% lower but at 12°C on the falling phase, the control fish were 58% lower than the cycling fish.

The sodium space of the cycling fish (Fig. 13) ranged from 70.8 ml/kg at 8°C to 33.2 ml/kg at 16°C on the rising phase of the cycle, reflecting a drop of 53% in the space estimate as the temperature rose from 8°C to 16°C. Sodium

Table I. Relative proportions of bound to unbound chloride in the plasma or rainbow trout and the effect of temperature on this binding.

Temperature	Sample size	Total chloride (Alk. Ext.)	Nitric Acetic Dilution Free Chloride	Ratio $\frac{\text{Free } Cl^-}{\text{Bound } Cl^-}$	Percent bound
4°C	10	Av. 75.4 (66.9-91.5)	Av. 63.5(53.3-66.7)	0.842	15.8%
12-15°C	10	Av. 102 (92-111)	Av. 101.6(91.4-110.4)	0.992	0.80%

Note: Values for total and free chloride are in mEq/L. Averages are given (Av.) as well as the range of values obtained.

space estimates are greater at the bottom of the temperature cycle, and smaller at the high temperatures of the cycle.

The sodium space in the control fish is also significantly higher at the low temperatures of the cycle. Values range from 74.9 ml/kg at 8°C to 49.0 ml/kg for fish held at 16°C, a decrease of 34%. In comparing the control fish to the cycling fish, it is found that the two differ significantly at 10°, 12°, and 16°C on the rising phase of the cycle. The cycling fish are never more than 38% lower than the control fish.

In the third estimate of ECFV, the chloride-potassium space, the cycling fish ranged from 68.0 ml/kg to 136.3 ml/kg, a 100% increase from 14°C on the rising phase, to 12°C on the falling phase (Fig. 14). Large fluctuations in the chloride potassium space estimates are caused mostly by changes in plasma chloride levels; a rise in plasma chloride causes a fall in the chloride-potassium space estimate.

In the control fish, the chloride-potassium space estimates do not differ significantly at the 5% level. Nevertheless, the controls differ significantly from the cycling fish at temperatures of 12° and 16°C on the rising phase of the cycle, and 14° and 12°C on the falling phase of the temperature cycle. In the control fish, changes in the chloride-potassium space reflect reciprocal changes in plasma chloride levels (compare Fig. 10 and 14).

Of the three space estimates in the cycling fish, the chloride space is the largest, and the sodium space the smallest. Table II shows a comparison between the three space

estimates, at all temperatures of the cycle, as well as a comparison between the controls held at different temperatures.

Table II. Percentage of the chloride space that is represented by the sodium space or the chloride-potassium space.

Cycling fish

Temperature °C	8	10	12	14	16	18	16	14	12	10
Sodium Space %	52	28	24	42	22	78	44	36	27	45
Chloride Potassium-% Space	53	77	76	54	78	64	50	73	68	58

Control fish

Temperature °C	8	10	12	14	16	18
Sodium Space %	54	45	65	62	50	46
Chloride Potassium-% Space	54	55	44	45	65	64

Figure 12 Changes which occur in the chloride space estimates of extracellular fluid volume in the cycling fish (solid line) and in the control fish (dashed line). Graphic presentation is the same as in Fig. 2.

F (cycling) $= 2.40 > 1.98_{.05}$

F (control) $= 5.85 > 2.38_{.05}$

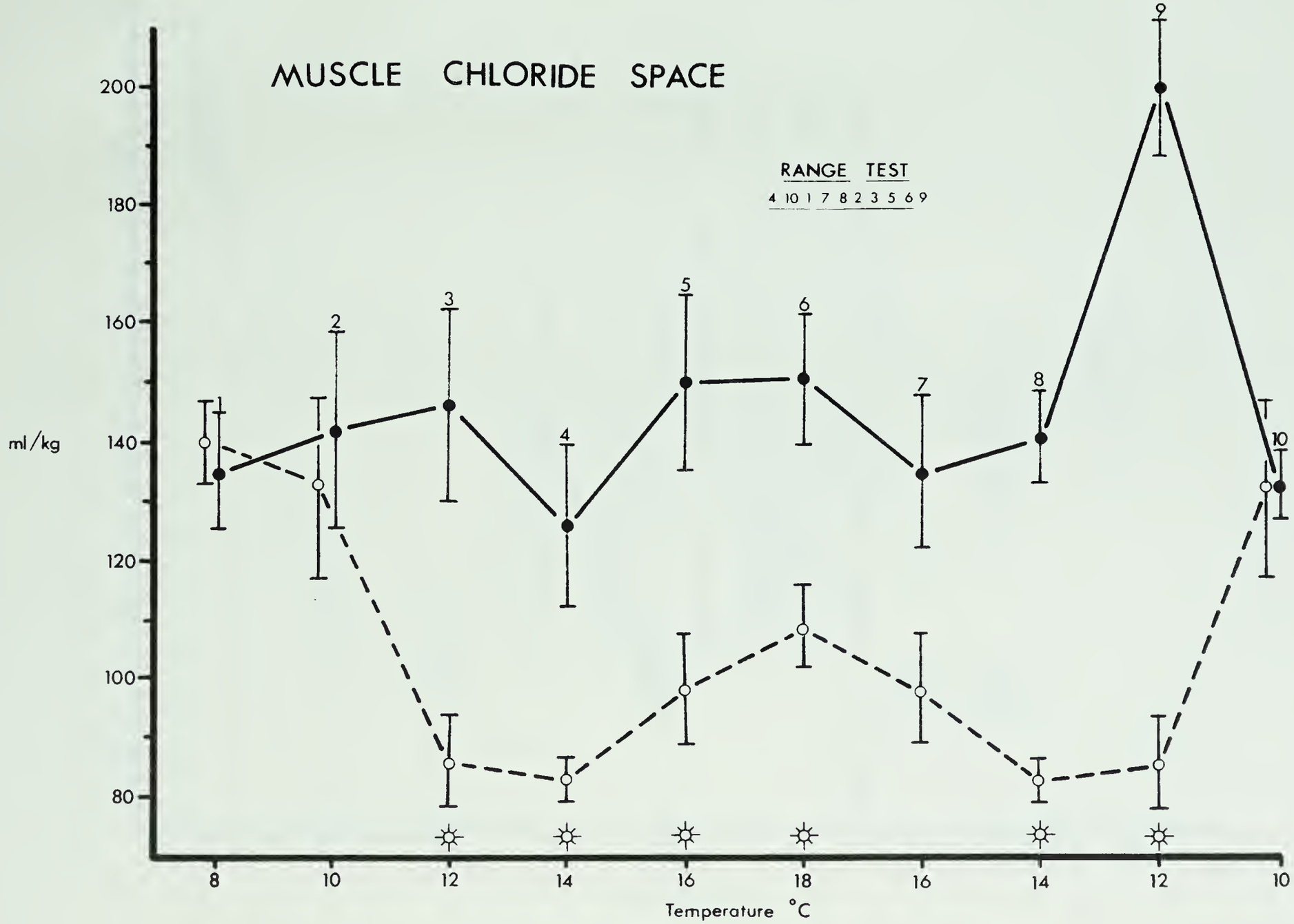

MUSCLE CHLORIDE SPACE
RANGE TEST
4 10 1 7 8 2 3 5 6 9
ml/kg
200
180
160
140
120
100
80
8
10
12
14
16
18
16
14
12
10
Temperature °C
1
2
3
4
5
6
7
8
9
10

Figure 13 Changes which occur in the sodium space estimates of extracellular fluid volume in the cycling fish (solid line) and in the control fish (dashed line). Graphic presentation is the same as in Fig. 2.

$F_{(cycling)} = 6.28 > 1.98_{.05}$

$F_{(control)} = 6.49 > 2.38_{.05}$

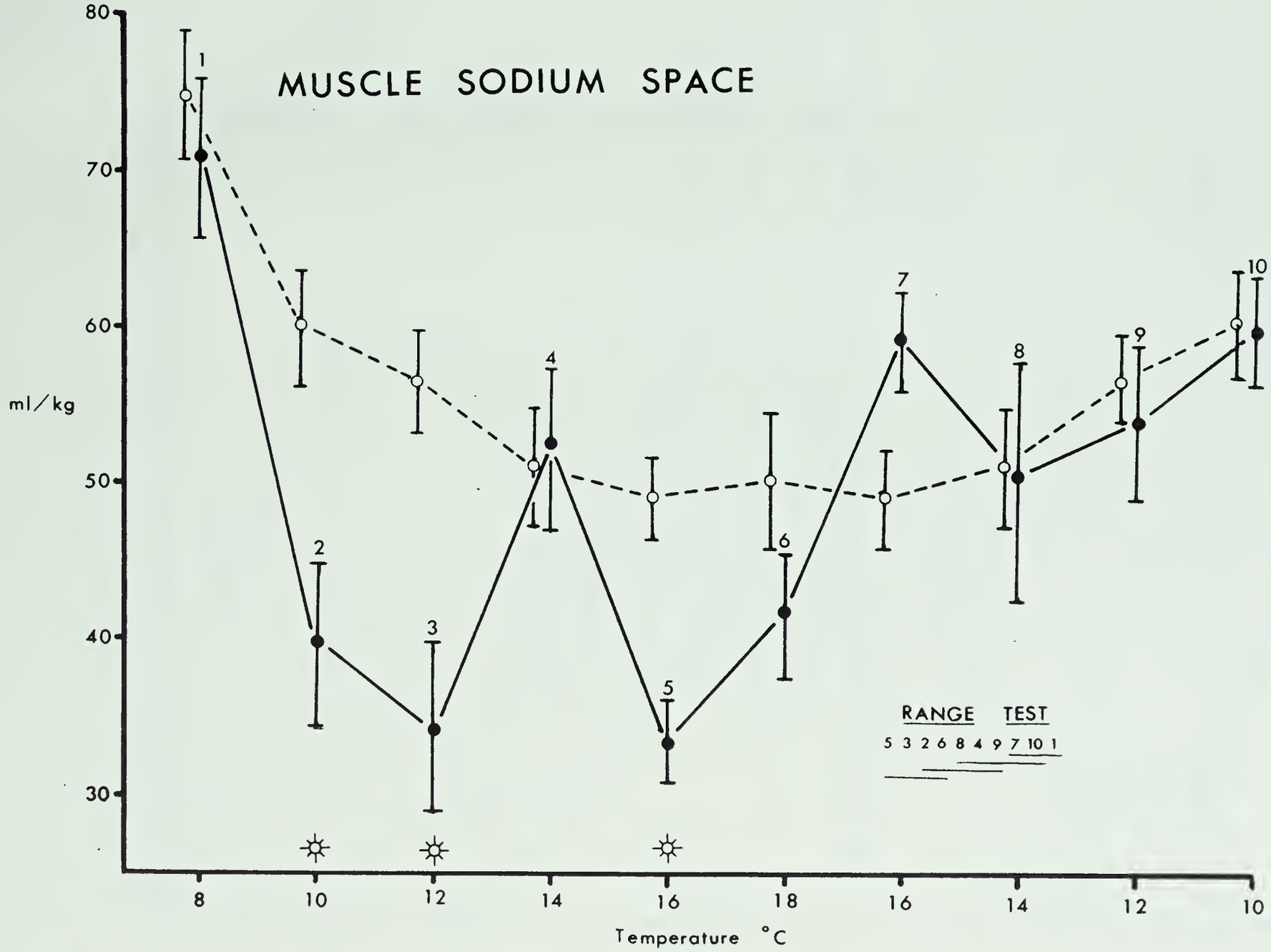
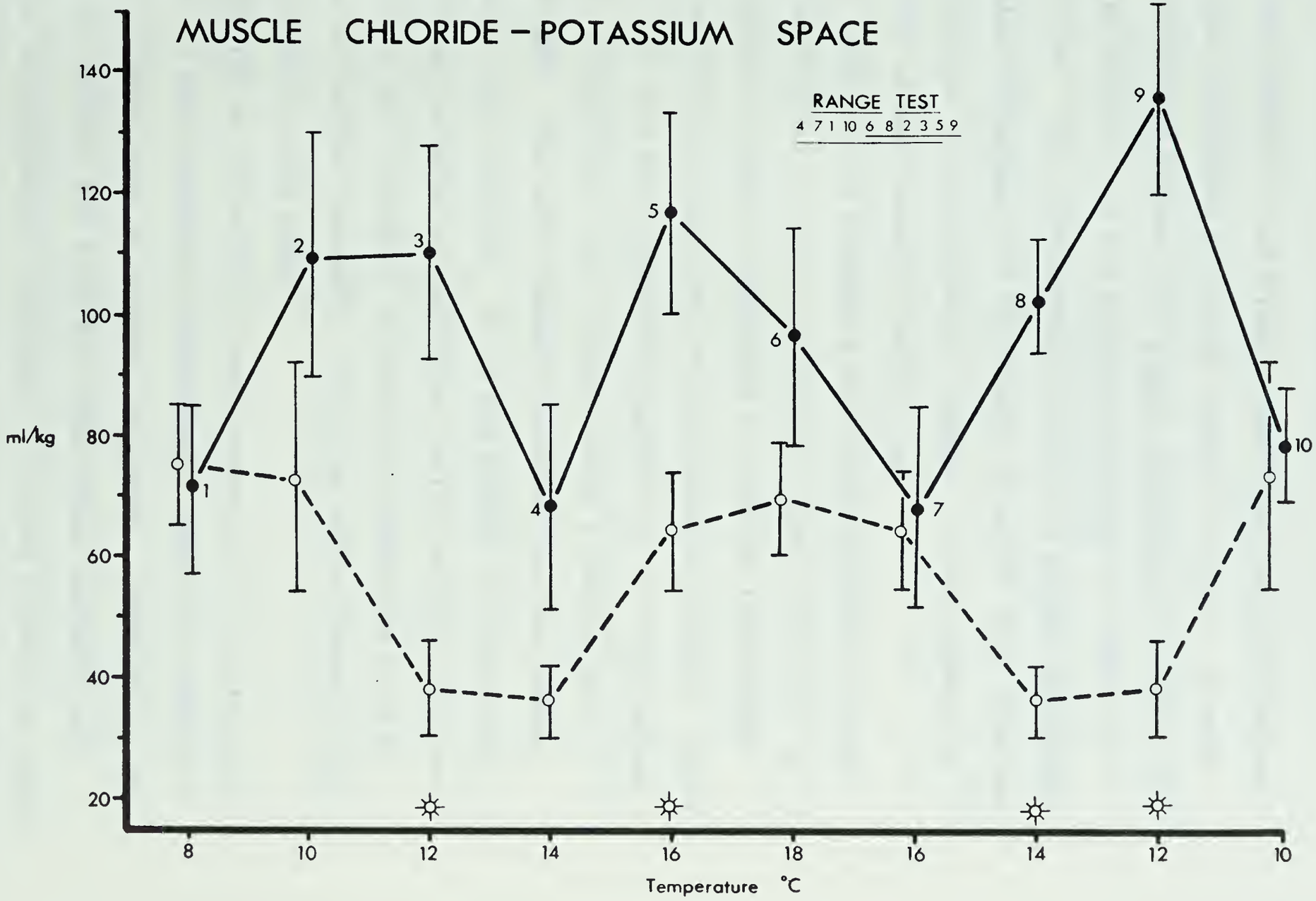
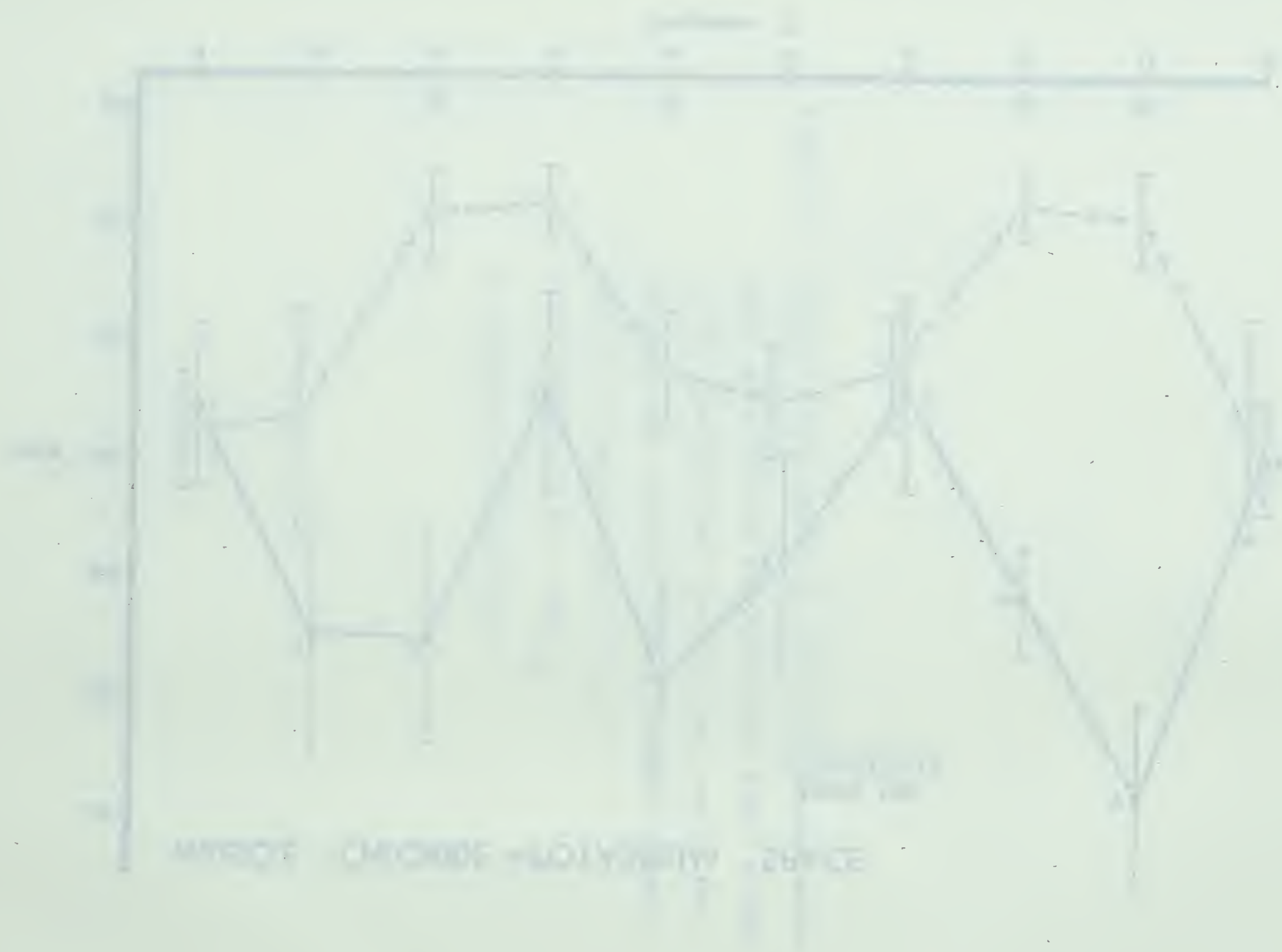

MUSCLE SODIUM SPACE
80
70
60
50
40
30
ml/kg
1
2
3
4
5
6
7
8
9
10
RANGE TEST
5 3 2 6 8 4 9 7 10 1
8
10
12
14
16
18
16
14
12
10
Temperature °C

Figure 14 Changes which occur in the chloride-potassium space estimates of extracellular fluid volume in the cycling fish (solid line) and in the control fish (dashed line). Graphic presentation is the same as in Fig. 2.

F (cycling) = $2.16 > 1.98_{.05}$

F (control) = 2.33 (N.S.)


MUSCLE CHLORIDE – POTASSIUM SPACE
RANGE TEST
4 7 1 10 6 8 2 3 5 9
ml/kg
140
120
100
80
60
40
20
1
2
3
4
5
6
7
8
9
10
8
10
12
14
16
18
16
14
12
10
Temperature °C



DISCUSSION

The concept of self-regulation in biological systems was first introduced by Claude Bernard in 1878. He made the distinction between the milieu exterieur, in which the organism exists, and the milieu interieur, in which all cells live. The relationship between the milieu interieur and milieu exterieur and the manner in which this relationship responds to environmental change, is one segment of the field of environmental physiology.

A study of the movements and concentrations of water and electrolytes in living organisms under conditions of environmental stress is expected to provide information about body fluid adaptation to environmental change. Water is, by far, the most abundant component of a living organism, in a freshwater fish, constituting 65-85 percent of body weight. The most abundant electrolytes are sodium, potassium, and chloride. It is difficult to excise and analyse the contents of individual cells and thus the concentrations and movements of cellular components must be studied by indirect methods, utilizing whole tissues, such as was done in this study.

Cycling or fluctuating temperatures are characteristic of much of the freshwater environment, especially smaller bodies of water such as streams and ponds. Populations of fishes do survive and flourish under these unsteady and rigorous environmental conditions. Yet the effects of rapid cyclic changes in temperature have only recently been investi-

gated, and virtually nothing is known of the effect of a cycling temperature on osmotic regulation and internal electrolyte composition.

Rainbow trout subjected to a daily cycling temperature (8° to 18°C daily) showed a small but significant increase in muscle water content as the temperature began to rise at the early part of the cycle. Throughout the rest of the temperature cycle, the water content of the muscle remained unchanged. In the control fish, on the other hand, there was a marked and significant decrease in muscle water at temperatures above 14°C. The cycling fish have muscle water concentrations nearly identical to those of the control fish held at the temperatures 8°, 10°, 12°, and 14°C. It is probable that the loss of tissue water in the controls is a long term adaptation to higher temperatures or the effect of increased growth rate (as indicated by an increased coefficient of condition) at higher temperatures. Decreases in muscle water content have been shown to result from increased growth by Houston and Threadgold (1963). The cycling fish would not show a similar water loss because of the relatively brief period of time spent at the high temperatures of the cycle.

Because the plasma water concentrations did not change significantly in the cycling fish, and the muscle water concentration was affected only slightly by the temperature cycle, it is unlikely that there was any temperature induced shift of muscle water to or from the environment.

This conclusion is also supported by the fact that the coefficient of condition did not change significantly in the cycling fish.

Hickman et al. (1964) found that short term exposure to cold temperature (one day) did not affect the coefficient of condition in rainbow trout but that after three days of cold exposure the condition factor rose significantly, indicating an 8.6% increase in body weight. It is apparent from my results that the cycling temperature did not cause any major shifts in plasma water, muscle water, on coefficient of condition.

Plasma sodium levels of trout maintained in a cycling temperature fluctuate greatly and do not show an obvious relationship to the temperature of the cycle. The rise in plasma sodium in the control fish at higher holding temperatures is on the other hand very significant. At higher temperatures it is possible that there is an increase in activity in the organs of exchange (primarily the gills and to a lesser extent the kidney) resulting in increased ammonia excretion in exchange for sodium. Maetz and Romeu (1964) have shown this ammonia-sodium exchange to take place in the goldfish gill, such that increased ammonia excretion could result in increased levels of sodium in the plasma. In this way, the increase in temperature resulting in an increase in metabolism, changes the balance of the metabolic constituents, which would have a direct effect on the levels of sodium in the plasma. The cycling fish did not show an increased plasma sodium concentration at the higher tempera-

tures of the cycle, but seem to have acclimated to levels similar to those of the control fish held at lower temperatures.

Plasma potassium levels in the cycling fish do not show any relationship to the temperature of the cycle. The variability within any sample however is greatly increased over that found in the control fish. In the control fish, plasma potassium levels are significantly lower at the high holding temperatures. This potassium decrease is probably a secondary effect of increased sodium in the plasma at the higher holding temperatures. In comparing the cycling fish with the control fish, it is again seen that the levels of the cycling fish approximate the levels of the control fish held at 8° and 10°C.

Plasma chloride levels in the cycling fish seldom exceed the levels found in the control fish at 8-10°C, indicating that the cycling fish are acclimating to the low end of the temperature cycle. These chloride levels do not show any apparent relationship to the temperature of the cycle but show a rising trend between 8-14°C on the rising phase of the cycle, and then tend to decrease for the remainder of the cycle.

Because large fluctuations in the plasma sodium and chloride levels are not reflected in fluctuations in cellular sodium and chloride, it is suggested that exchange of plasma sodium and chloride during the temperature cycle are made directly with the environment, in all probability via the gills.

The exchange of sodium and chloride is not apparently related as shown by the lack of relationship between these two ions in the plasma. Romeu and Maetz (1964) have also shown that in the goldfish (Carassius auratus), there is an independent uptake of sodium and chloride ions, and that in both cases the uptake is an active process. It has been suggested by Ussing (1954) and subsequently proved, that sodium is actively taken up by the frog skin, and that chloride ions follow passively. Passive chloride uptake in response to a sodium gradient seems unlikely in rainbow trout because of the independent behavior of the sodium and chloride in response to temperature change.

In the control fish, an even more interesting relationship occurs between these two ions. Plasma sodium concentrations increase significantly as the holding temperature is increased, whereas the plasma chloride concentrations are low at 8° and 19°C, high at 12° and 14°C and then drop significantly at 16° and 18°C (compare Fig. 5 and 10). With the sodium concentration at a high level at 16° and 18°C, the decrease in plasma chloride at these temperatures produces a great excess of sodium to chloride and making necessary a substantial increase in some other anion to balance sodium. Because bicarbonate (arising from metabolism) has been shown to exchange for chloride across the goldfish gill (Maetz and Romeu 1964), it is possible that bicarbonate is the anion which is replacing the chloride in the plasma at high temperatures.

Decreased levels of chloride in the plasma, such as those

which occurred at 16° and 18°C in the control fish could have resulted from a compensatory rise in the bicarbonate level in the plasma. As the CO_2 in the plasma is readily removed, the high bicarbonate concentration would result in a state of alkalosis, which in turn could cause ion rearrangement by removing potassium from the muscle (Elkinton and Danowski 1955). The lower levels of muscle potassium in the control fish at higher temperatures support this explanation to some extent. In the cycling fish however, the lowest level of chloride in the plasma (and presumably the highest bicarbonate level) is at 12°C on the falling phase of the cycle, but the muscle potassium level at this point is not at its lowest level. It seems unlikely that alkalosis is affecting potassium muscle levels in the cycling fish to any significant extent.

Muscle sodium and potassium levels in the cycling fish do not show any noticeable relationship to the temperature of the cycle although muscle sodium levels show a definite reciprocal relationship to the muscle potassium levels (Fig. 9). It is quite possible that decreased sodium extrusion at various points of the cycle resulted in potassium leaking out of the cell. At temperatures of the cycle where muscle sodium levels are high, the corresponding muscle potassium levels are very low. This is an even more interesting change when it is noticed that the control fish levels of muscle sodium and potassium are not significantly affected by the holding temperature. It appears therefore, if acclimation to the prevailing temperature is complete, muscle sodium

and potassium concentrations will attain similar values, at least within the temperature range of 8-18°C. This inverse relationship between the muscle sodium and potassium ions seen in the cycling fish has been demonstrated in the squid axoplasm by Steinbach and Spiegelman (1943) and more recently in muscle by Cotlove et al. (1951). In contrast to my results, Hickman et al. (1964) have shown that trout held at a low temperature resulted in the loss of both sodium and potassium from the muscle.

Very little change occurred in the muscle chloride concentrations in the cycling fish inspite of large fluctuations in the plasma chloride levels. Muscle chloride in the control fish was also relatively steady, showing a small but insignificant rise at 18°C. This constancy in muscle chloride levels may indicate that muscle chloride does not exist in the free ionic form but is in some way bound, perhaps to intracellular protein. Daniel (1958) found that in smooth muscle the chloride space was larger than the sodium space (as was found in my results), with the suggestion that the intracellular chloride was bound in a non-ionic form. The method used to extract muscle chloride was the alkaline digestion method (Cotlove 1963b) which completely frees all chloride present, therefore the bound as well as the unbound chloride would be measured coulometrically in its ionic form.

It has been suggested by Cotlove and Hogben (1962) and Conway (1957) that chloride and potassium are distributed across the cell membrane according to a Donnan distribution.

If this were true in the rainbow trout, the changes in plasma chloride levels in the controls as well as the cycling fish would be partially reflected in the muscle chloride levels. It is possible to conclude that chloride and potassium ions do not distribute themselves simply as in a Donnan equilibrium in rainbow trout.

It is important to note that the Gibbs-Donnan law on the distribution of ions was originally applied to a simple system in which the protein molecules were thought of as non-diffusible ions which did not influence the distribution of other ions in the system, other than by their nondiffusible nature. In biological systems it is now well known that the protein present influences the distribution of ions in many complex ways.

All plasma ions, even those which are univalent, appear to be bound to an appreciable degree by plasma albumin. The tenacity of the binding is so great that in ordinary buffer solutions containing only univalent ions, the binding of the anions completely dominates cation binding and the latter can be ignored except in strongly alkaline solutions (Foster 1960).

Carr (1952) has shown that serum proteins bind chloride to an appreciable degree at low pH values. Thirty chloride ions were bound to one mole of crystalline bovine albumin at pH 3.0. Under more physiological conditions of pH 7.4 it was found that the binding of chloride was 3.0 mEq/L of serum, which would decrease the amount of diffusible chloride by approximately 3%. Carr also concluded that the relative

binding affinities of the following ions increase in the following order; chloride < bromide < nitrate < iodide.

Recent work by a graduate student at the University of Alberta, Mr. Chau-ting Huang (pers. comm.) has shown that approximately 80% of the iodide ions in the rainbow trout plasma are bound to the serum ablumin. To a lesser extent he has found protein bound inorganic iodide in the northern pike (Esox lucius), and little, if any, binding in the white sucker (Catostomus commersonii).

Meisner and Hickman (1962) have shown that cold acclimation increases the level of serum albumin in the blood of rainbow trout. If the amount of serum albumin increased at lower temperatures, one could suspect that the amount of anionic binding would also increase at the lower temperatures.

From my results it was determined that 15% of the chloride present in the plasma at 4°C was bound, quite possibly to the serum albumin. At acclimation temperatures of 12-15°C however, there was no indication of chloride binding. The type of chloride bond would probably be of a strong chemical nature, as opposed to the weak type of bond which iodide has been shown to make (Huang pers. comm.).

Several possible explanations may be advanced for the increased binding at low temperatures. First, low temperatures could increase the total amount of serum albumin in the plasma, as shown by Meisner and Hickman (1962), making more binding sites available. A second possible explanation for increased binding is found in the work by Carr (1952) which shows that chloride binding increases as the pH decreases.

As the pH decreases and the net positive charge on the protein molecule increases, the electrostatic attraction for small anions increases. Low temperatures in my trout could have resulted in a lower plasma pH (which was not measured in this study) resulting in an increase in chloride binding and a consequential decrease in free chloride levels. As has been mentioned previously, consistent with this possibility, alkalosis may have been present at higher temperatures. The third, and probably most important, cause for increased binding is that there is a greater attraction of binding sites at low temperatures (Maron and Prutton 1958).

The possible effects of binding are shown by the work of Houston (1962) who showed that in the goldfish, free plasma chloride was lost when the fish were held at low temperatures. Hickman et al. (1964) also showed that in the rainbow trout the initial response to cold temperature was a resultant drop in plasma chloride levels. The control fish in my study also show low levels of plasma chloride when held at low temperatures, possibly an effect of increased binding or also a decrease in the bicarbonate-chloride exchanges in the gills.

The use of the various space estimates for determining the concentration of the extracellular fluid volume (ECFV) has been questioned by several researchers. Manery (1954) recognizes the limitations of using the Donnan factors in calculating space estimates in biological material. Gordon (1959) criticizes the use of any space estimate of ECFV which is used to determine absolute volumes. He suggests

that estimates of ECFV are useful only if relative changes are desired. The usefulness of these estimates to indicate relative changes can even be questioned, on the basis of my experiments. Other substances, such as inulin, have been infused prior to the obtaining of a tissue specimen, and then have been utilized for the reference point for the ECFV. However, the validity of the inulin space as a measurement of ECFV has been questioned and to date this problem of an adequate substance for ECFV measurement has not been solved (Page 1962).

Because chloride is not entirely confined to the extracellular fluid, the chloride space clearly overestimates the value for ECFV. However, the fact that the chloride space is larger than the sodium space suggests that the intracellular chloride is bound to an intracellular moiety.

The chloride-potassium space estimate of ECFV (after Conway 1957) would not be useful in the rainbow trout in view of the fact that potassium and chloride are not distributed across the cell membrane in a simple Donnan fashion. The fact that no compensatory changes occurred in the muscle potassium and chloride after large fluctuations in the plasma, support this fact.

In both the chloride space and the chloride-potassium space, the relatively high estimates of ECFV reflect the low levels of plasma chloride concentrations in the cycling fish, and the control fish held at temperatures below 14°C.

When the intracellular concentrations of sodium were

made using the chloride space as the estimate of ECFV (formulae after Manery 1954) only negative values were obtained. When the chloride-potassium space was used as the estimate of ECFV, negative concentrations occurred approximately one-half of the time. It is apparent that both space estimates do not give reliable estimates of ECFV in rainbow trout, and would be of limited use in discussing body fluid and electrolyte movements in these fish.

An important point to observe in the space estimates is that in almost all cases, in the cycling fish and the control fish, the sodium space is the smallest space estimate. The probable reason for this is that chloride diffuses or is transported into the cell more than sodium. Thus, unless some of the plasma sodium is bound, the ECFV must at least be no larger than the sodium space.

If the sodium space is used to give an indication of ECFV changes, it can be seen that in both the cycling fish and the controls, high temperatures significantly decrease the ECFV. In the control fish, the increase in ECFV at lower temperatures and the change to a lower ECFV at high temperatures, is a uniform one, decreasing at each temperature as the temperature rises. In the cycling fish the variability in sodium space is great, and changes in the space estimate reflect very closely changes in tissue sodium levels.

It is not suggested that the formulae used to calculate ECFV and intracellular concentrations are wrong, but the underlying assumptions are of questionable value when applied to rainbow trout. It appears that the trout can withstand

drastic changes in electrolyte composition which would normally be harmful to mammalian tissue. The great amount of variability has frequently been recorded in other species of fish (Houston 1959, Parry 1961, and Houston 1962).

One of the most notable findings of this study was that the measured electrolyte concentrations of the cycling fish more closely resembled the levels measured in the cold acclimated than in the warm acclimated fish. The question arises as to whether or not this resemblance represents acclimation to the low temperatures of the cycle. Acclimation to an environmental variable is generally considered to represent a definable physiological response to a change in an environmental parameter to a new level. In the present study however, the environmental variable -temperature- was never constant. It is possible that the trout did not acclimate to the low temperature in the usually accepted sense but that the measured response, in which a steady state condition was never attained, represents a physiological compromise to the conditions. To demonstrate this point conclusively, a type of "sine wave" temperature cycle would have to be designed in which the fish experienced equal times at all temperatures.

Why did the trout appear to adjust to the low end of the temperature cycle? As mentioned previously, because a longer period of time was spent at the low temperatures, this may have been the logical response to expect. It may also be possible that physiological processes such as the sodium pump may be more efficient if "geared" to a lower temperature and

then required to become more active as the temperature rises, rather than the other way around.

Nevertheless, to obtain a complete understanding of the physiological changes taking place in a fish in a cycling regime, one would have to follow not only sodium, potassium, chloride, and water changes but also other constituents in the plasma, muscle and perhaps other tissues, as well as the effects of general activity on electrolyte metabolism. Knowledge of fluctuations in bicarbonate levels, pH changes, plasma protein concentration changes, and an accurate estimate of ECFV would all be very desirable. An index of general metabolism (such as oxygen consumption) would have also added much to this study.

SUMMARY

1. One and one-half year old rainbow trout, Salmo gairdneri, were acclimated to a daily temperature cycle ranging from 8° to 18°C. Control fish were maintained at constant temperatures which coincided with the sample temperatures of the cycling fish.

2. Rainbow trout acclimated to temperatures above 14°C show a significant water loss from the muscle whereas the cycling fish do not lose muscle water while passing through the higher temperatures of the cycle.

3. Plasma water content in both the cycling fish and the control fish did not change significantly at any temperature of the cycle or at any holding temperature.

4. Plasma chloride in the cycling fish increased between 8°C and 14°C on the rising phase of the cycle, then decreased for the remainder of the cycle. Plasma chloride levels of the cycling fish are similar to those of the control fish held at lower temperatures (8-10°C).

5. Muscle chloride is relatively stable in both the cycling trout and controls. It was suggested that very little of the chloride is found in the cells and that the chloride present there may be bound to intracellular proteins and consequently not freely diffusible. If so, chloride would not be distributed as in a simple Donnan equilibrium.

6. Plasma sodium and potassium levels in the cycling fish, are similar to the plasma sodium and potassium levels in control fish held at 8°C and 10°C.

7. There is a highly significant inverse correlation between the muscle potassium and the muscle sodium levels in the cycling fish. Muscle sodium and potassium levels in the control fish do not exhibit this inverse relationship.

8. The chloride space and the chloride-potassium space were found to be poor estimates of extracellular fluid volume in the rainbow trout. The sodium space, the lowest space estimate, indicated that in both the cycling and control fish the extracellular fluid volume increased significantly as temperature increased (holding temperature or cycling temperature).

9. Temperature dependent chloride binding was found in rainbow trout. Fish acclimated to 4°C had 15.8% bound plasma chloride whereas trout acclimated to 12-15°C did not show plasma chloride binding.

10. Evidence from plasma and tissue concentrations of electrolytes in both the cycling and control trout indicated that cycling trout acclimated to the lowest temperatures of the cycle.

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APPENDIX I

Formulae used to calculate the chloride space, sodium space, and the chloride-potassium space. The Donnan factors used were provided by Manery (1954). The chloride-potassium space estimate of extracellular fluid volume was first provided by Conway (1957) and contained two typological errors which were subsequently corrected at the end of the January 1958 issue of Physiological Reviews. Subscripts t, p, and e, denote concentrations in whole tissue, plasma, and extracellular water respectively.

$$\text{Cl space} = \frac{Cl_t \times .977 \times (H_2O)_p}{Cl_p}$$

$$\text{Na space} = \frac{Na_t \times (H_2O)_p}{Na_p \times .942}$$

$$\text{Cl-K space} = \frac{K_t Cl_t - ((H_2O)_t)^2 Cl_e K_e}{K_e Cl_e + Cl_t K_e - 2(H_2O)_t Cl_e K_e}$$

where $Na_e = Na_p \times .942/(H_2O)_p$

and $K_e = K_p \times .943/(H_2O)_p$

Estimates of extracellular fluid volume, Na space, Cl space, and Cl-K space are in litres per kilogram of muscle.

APPENDIX II

Data from individual fish

Key to tables on following pages:

TEMP = temperature in degrees centigrade.

WT. = fish weight in grams.

LTH. = fish length in centimetres.

C.C. = coefficient of condition.

NA P = plasma sodium in mEq/L.

K P = plasma potassium in mEq/L.

CL P = plasma chloride in mEq/L.

H2OP = plasma water in litres/kg.

NA T = muscle sodium in mEq/kg wet tissue.

K T = muscle potassium in mEq/kg wet tissue.

CL T = muscle chloride in mEq/kg wet tissue

H2OT = muscle water in litres/kg.

CL SP = muscle chloride space in litres/kg.

NA SP = muscle sodium space in litres/kg.

CLK SP = muscle chloride-potassium space in litres/kg.

DATA FROM INDIVIDUAL FISH

MEANS ARE GIVEN AT THE BOTTOM OF EACH TEN FISH

U=RISING PHASE D=FALLING PHASE C=CONTROL FISH

TEMP	WT.	LTH.	C.C.	NA P	K P	CL P	H2O P	NA T	K T	CL T	H2O T	CL SP	NA SP	CLK SP
U 8.0	30.0	14.6	0.96	110.	4.5	107.1	0.9750	6.8	63.8	8.83	0.7710	0.0846	0.0634	0.0486
U 8.0	21.7	13.0	0.96	105.	3.8	70.9	0.9740	6.5	61.2	8.44	0.7880	0.1220	0.0635	0.0926
U 8.0	38.1	16.2	0.91	140.	4.8	59.6	0.9870	6.6	62.1	9.97	0.7920	0.1738	0.0490	0.1420
U 8.0	23.1	12.7	1.13	90.	5.3	69.6	0.9440	6.9	63.4	8.46	0.7700	0.1208	0.0762	0.0805
U 8.0	24.5	13.5	1.00	75.	9.7	81.0	0.9730	7.6	90.3	9.74	0.7750	0.1232	0.1038	0.0708
U 8.0	23.7	13.4	0.99	90.	9.5	78.2	0.9740	7.0	74.6	7.65	0.7940	0.1003	0.0797	0.0270
U 8.0	23.5	13.2	1.02	100.	13.4	68.0	0.9640	7.1	72.0	8.41	0.7870	0.1255	0.0720	0.0162
U 8.0	27.5	13.5	1.11	116.	14.4	58.2	0.9730	8.7	64.0	8.03	0.7810	0.1413	0.0768	0.0106
U 8.0	28.2	13.7	1.10	90.	8.0	59.9	0.9880	6.2	58.1	9.36	0.7940	0.1625	0.0716	0.0974
U 8.0	25.5	13.5	1.04	130.	11.8	45.4	0.9880	6.5	69.0	8.57	0.7830	0.1963	0.0520	0.1233
	MEANS		1.02	105.	8.5	69.8	0.9740	7.0	67.8	8.75	0.7835	0.1350	0.0708	0.0709
U10.0	40.4	13.6	1.60	85.	7.2	45.9	0.9810	2.2	81.0	9.46	0.7940	0.2128	0.0267	0.1798
U10.0	35.4	15.5	0.95	65.	7.6	38.1	0.9770	2.4	94.0	9.66	0.7920	0.2607	0.0380	0.2360
U10.0	42.2	16.4	0.96	120.	5.3	60.9	0.9660	4.4	87.6	9.97	0.8090	0.1665	0.0373	0.1396
U10.0	41.3	15.6	1.09	100.	7.8	102.5	0.9710	3.1	82.5	8.70	0.7880	0.0868	0.0317	0.0340
U10.0	51.2	16.6	1.11	100.	4.5	71.5	0.9820	2.5	89.6	10.25	0.7860	0.1482	0.0258	0.1270
U10.0	40.5	16.3	0.94	85.	4.8	88.2	0.9730	3.5	89.1	10.28	0.7990	0.1194	0.0422	0.0930
U10.0	45.7	16.5	1.02	65.	4.0	90.1	0.9870	2.2	97.1	8.70	0.7940	0.1003	0.0352	0.0800
U10.0	49.2	16.8	1.04	116.	4.8	89.9	0.9600	2.4	92.2	9.22	0.7910	0.1036	0.0209	0.0773
U10.0	33.5	14.0	1.22	74.	4.8	71.6	0.9690	5.1	75.5	8.50	0.7920	0.1211	0.0703	0.0902
U10.0	35.4	15.0	1.05	90.	7.1	81.4	0.9600	6.1	63.6	8.43	0.7990	0.1047	0.0685	0.0409
	MEANS		1.10	90.	5.8	74.0	0.9726	3.4	85.2	9.32	0.7944	0.1424	0.0397	0.1098
U12.0	46.1	16.7	0.98	145.	9.8	51.0	0.9870	2.5	93.2	9.64	0.7960	0.1964	0.0179	0.1540
U12.0	50.5	17.6	0.93	95.	5.5	82.9	0.9660	3.7	103.7	8.82	0.7950	0.1082	0.0396	0.0815
U12.0	46.6	18.0	0.79	170.	6.9	47.5	0.9780	4.6	97.3	11.12	0.8030	0.2410	0.0279	0.2170
U12.0	40.0	16.1	0.96	95.	7.3	51.9	0.9870	3.4	100.9	10.11	0.7950	0.2024	0.0372	0.1753
U12.0	36.0	15.2	1.03	90.	8.9	84.3	0.9600	2.0	80.2	9.18	0.7970	0.1100	0.0225	0.0481
U12.0	34.5	14.7	1.09	116.	6.7	64.2	0.9640	3.3	96.5	9.34	0.7910	0.1476	0.0289	0.1162
U12.0	36.9	15.7	0.95	95.	7.8	89.4	0.9650	4.0	89.6	10.40	0.7970	0.1182	0.0428	0.0730
U12.0	38.8	14.9	1.17	95.	4.8	117.8	0.9650	2.7	94.6	8.48	0.7920	0.0731	0.0289	0.0452
U12.0	47.8	16.6	1.04	116.	5.8	60.5	0.9800	2.4	85.2	8.84	0.8060	0.1507	0.0213	0.1194
U12.0	29.2	14.1	1.04	85.	5.0	88.7	0.9760	6.4	66.7	9.80	0.7890	0.1135	0.0774	0.0762
	MEANS		1.00	110.	6.8	73.8	0.9728	3.5	90.8	9.57	0.7961	0.1461	0.0344	0.1106

DATA FROM INDIVIDUAL FISH

MEANS ARE GIVEN AT THE BOTTOM OF EACH TEN FISH

U=RISING PHASE D=FALLING PHASE C=CONTROL FISH

TEMP	WT.	LTH.	C.C.	NA P	K P	CL P	H2O P	NA T	K T	CL T	H2O T	CL SP	NA SP	CLK SP
U14.0	50.8	17.0	1.03	100.	10.4	133.1	0.9890	3.0	97.1	9.56	0.7930	0.0748	0.0312	0.0119
U14.0	38.0	15.7	0.98	100.	6.9	92.3	0.9520	4.1	93.2	10.19	0.7980	0.1106	0.0411	0.0714
U14.0	37.0	14.8	1.14	75.	4.2	70.7	0.9560	2.3	79.8	10.41	0.7940	0.1482	0.0309	0.1246
U14.0	42.5	16.0	1.04	140.	9.6	99.3	0.9840	5.7	70.5	9.68	0.7960	0.1010	0.0422	0.0220
U14.0	34.5	14.5	1.13	105.	5.3	109.3	0.9710	5.1	69.7	8.49	0.7910	0.0794	0.0496	0.0370
U14.0	35.2	15.5	0.95	90.	7.8	59.7	0.9810	6.8	62.7	10.09	0.7930	0.1745	0.0780	0.1190
U14.0	26.5	14.0	0.97	100.	8.5	81.5	0.9740	6.0	65.4	9.51	0.7860	0.1196	0.0615	0.0506
U14.0	40.7	16.5	0.90	120.	8.5	48.3	0.9810	6.3	57.7	10.84	0.7940	0.2318	0.0542	0.1758
U14.0	32.1	14.2	1.12	90.	7.8	92.5	0.9660	5.8	68.4	9.93	0.7960	0.1092	0.0655	0.0457
U14.0	35.8	15.6	0.94	95.	9.5	71.1	0.9880	6.4	58.7	7.93	0.7980	0.1160	0.0701	0.0220
	MEANS		1.02	102.	7.8	85.8	0.9742	5.1	72.3	9.66	0.7939	0.1265	0.0524	0.0680
U16.0	40.0	15.0	1.18	80.	5.3	81.6	0.9730	3.0	98.2	8.76	0.7810	0.1100	0.0384	0.0843
U16.0	31.0	14.7	0.98	105.	5.0	80.8	0.9650	4.4	89.9	10.59	0.8070	0.1331	0.0426	0.1061
U16.0	45.5	16.3	1.05	120.	8.7	71.7	0.9700	2.2	79.5	8.32	0.7940	0.1185	0.0187	0.0604
U16.0	49.2	16.5	1.10	116.	7.6	37.9	0.9600	3.0	93.8	9.12	0.8010	0.2432	0.0261	0.2152
U16.0	30.0	14.1	1.07	65.	3.6	92.5	0.9730	2.6	84.2	7.84	0.7930	0.0868	0.0410	0.0646
U16.0	27.2	14.1	0.97	65.	4.0	81.8	0.9610	2.4	89.2	10.53	0.7930	0.1302	0.0374	0.1094
U16.0	47.4	17.6	0.87	116.	9.1	49.4	0.9720	3.4	100.1	10.08	0.8000	0.2088	0.0300	0.1733
U16.0	31.1	14.1	1.11	90.	9.1	68.4	0.9730	3.6	88.5	9.82	0.7940	0.1470	0.0410	0.0979
U16.0	30.0	14.1	1.07	90.	8.0	76.7	0.9600	2.5	90.2	10.28	0.7890	0.1354	0.0281	0.0925
U16.0	51.2	17.0	1.04	120.	5.8	54.2	0.9720	3.4	91.8	10.06	0.8000	0.1899	0.0290	0.1647
	MEANS		1.04	97.	6.6	69.5	0.9679	3.0	90.5	9.54	0.7952	0.1503	0.0332	0.1168
U18.0	48.6	17.7	0.88	130.	7.8	67.3	0.9790	4.3	94.0	9.55	0.8030	0.1462	0.0341	0.1069
U18.0	52.8	17.6	0.97	125.	7.3	42.6	0.9830	3.4	96.5	10.11	0.7930	0.2456	0.0281	0.2213
U18.0	41.3	16.0	1.00	75.	6.7	78.3	0.9680	3.1	96.2	9.81	0.7950	0.1277	0.0421	0.0938
U18.0	39.1	15.5	1.05	140.	7.6	57.4	0.9650	3.0	103.2	9.82	0.7970	0.1738	0.0218	0.1424
U18.0	38.0	15.7	0.98	90.	7.6	61.2	0.9550	3.4	95.8	9.34	0.7970	0.1534	0.0380	0.1165
U18.0	34.5	15.0	1.02	105.	11.1	75.8	0.9770	5.5	66.5	8.80	0.7920	0.1194	0.0539	0.0231
U18.0	36.0	15.7	0.93	135.	8.9	76.0	0.9810	6.7	64.6	9.10	0.7950	0.1236	0.0512	0.0489
U18.0	49.3	16.5	1.09	140.	9.6	67.2	0.9640	5.4	76.5	9.12	0.7920	0.1377	0.0391	0.0732
U18.0	33.5	15.7	0.86	105.	8.3	59.3	0.9750	6.4	60.0	9.35	0.7930	0.1618	0.0626	0.0954
U18.0	36.8	15.6	0.96	140.	8.2	83.1	0.9790	6.5	54.5	10.08	0.7910	0.1250	0.0478	0.0427
	MEANS		0.97	119.	8.3	66.8	0.9726	4.8	80.8	9.51	0.7948	0.1514	0.0419	0.0964

DATA FROM INDIVIDUAL FISH

MEANS ARE GIVEN AT THE BOTTOM OF EACH TEN FISH

U=RISING PHASE D=FALLING PHASE C=CONTROL FISH

TEMP	WT.	LTH.	C.C.	NA P	K P	CL P	H2O P	NA T	K T	CL T	H2O T	CL SP	NA SP	CLK SP
D16.0	33.2	14.7	1.04	105.	7.3	43.2	0.9800	6.6	62.4	9.98	0.7920	0.2383	0.0648	0.1973
D16.0	27.5	13.7	1.07	85.	6.0	48.5	0.9700	6.4	62.1	8.05	0.8000	0.1695	0.0769	0.1260
D16.0	29.7	14.8	0.92	110.	5.8	87.7	0.9720	6.8	68.0	9.22	0.7850	0.1076	0.0632	0.0640
D16.0	28.2	14.5	0.92	115.	6.5	87.8	0.9860	6.1	60.7	10.41	0.8010	0.1231	0.0551	0.0671
D16.0	33.3	15.0	0.98	130.	5.4	72.7	0.9730	6.0	55.7	9.28	0.7970	0.1307	0.0473	0.0820
D16.0	31.5	14.7	0.99	116.	8.0	79.2	0.9730	6.6	51.6	9.05	0.7910	0.1170	0.0583	0.0287
D16.0	23.3	13.3	0.99	90.	8.2	74.7	0.9800	6.1	67.7	7.61	0.7860	0.1051	0.0699	0.0393
D16.0	34.7	15.0	1.03	130.	9.5	74.0	0.9760	7.0	54.6	8.44	0.7860	0.1172	0.0553	0.0166
D16.0	29.5	14.5	0.97	130.	8.0	84.4	0.9910	7.3	52.3	8.85	0.7990	0.1094	0.0586	0.0202
D16.0	47.1	16.3	1.08	155.	8.3	68.5	0.9910	6.5	48.1	8.90	0.7990	0.1355	0.0437	0.0395
	MEANS		1.00	117.	7.3	72.1	0.9792	6.5	58.3	8.98	0.7936	0.1353	0.0593	0.0681
D14.0	35.7	14.7	1.17	45.	6.0	61.7	0.9860	3.3	97.8	9.81	0.7890	0.1650	0.0761	0.1399
D14.0	43.0	16.2	1.01	75.	6.7	87.1	0.9711	3.6	90.9	8.01	0.7920	0.0940	0.0491	0.0546
D14.0	38.8	15.3	1.08	105.	8.2	62.1	0.9610	3.2	89.0	8.72	0.7930	0.1420	0.0308	0.0976
D14.0	59.5	17.8	1.06	150.	9.5	48.5	0.9570	3.1	96.9	8.77	0.7940	0.1822	0.0208	0.1399
D14.0	51.5	17.2	1.01	95.	8.0	75.6	0.9550	4.4	77.9	8.47	0.7980	0.1126	0.0466	0.0561
D14.0	33.7	14.4	1.13	90.	5.8	64.9	0.9840	4.2	89.4	9.31	0.7930	0.1486	0.0483	0.1200
D14.0	31.6	14.5	1.04	116.	5.8	70.8	0.9600	3.9	93.9	9.18	0.8030	0.1310	0.0340	0.1007
D14.0	46.7	16.3	1.08	85.	4.0	65.2	0.9550	2.8	88.9	8.34	0.7960	0.1286	0.0331	0.1073
D14.0	22.7	12.8	1.08	95.	7.1	69.6	0.9720	5.7	72.0	10.99	0.7920	0.1616	0.0614	0.1172
D14.0	25.0	13.5	1.02	85.	6.2	63.9	0.9720	8.5	55.0	9.23	0.7870	0.1478	0.1023	0.0944
	MEANS		1.07	94.	6.7	66.9	0.9673	4.3	85.2	9.08	0.7937	0.1413	0.0503	0.1028
D12.0	46.8	17.0	0.95	115.	4.5	52.7	0.9650	4.6	98.3	10.87	0.7940	0.2095	0.0406	0.1931
D12.0	38.8	15.1	1.13	116.	10.1	59.1	0.9800	3.6	97.6	9.15	0.7960	0.1597	0.0320	0.1123
D12.0	39.7	16.0	0.97	95.	8.2	75.0	0.9500	3.5	99.9	8.67	0.7910	0.1156	0.0368	0.0730
D12.0	38.7	15.5	1.04	90.	6.2	54.6	0.9820	4.3	86.5	8.60	0.7930	0.1628	0.0494	0.1323
D12.0	39.7	15.8	1.01	85.	4.0	43.2	0.9550	6.1	63.5	9.62	0.7960	0.2239	0.0721	0.2014
D12.0	37.5	15.5	1.01	125.	8.9	47.0	0.9810	6.9	63.2	10.71	0.8040	0.2353	0.0570	0.1810
D12.0	23.5	13.4	0.98	100.	12.7	43.1	0.9910	6.4	55.6	8.19	0.7990	0.1982	0.0668	0.0843
D12.0	25.9	13.7	1.01	100.	22.8	43.1	0.9760	7.8	63.2	9.77	0.7660	0.2329	0.0801	0.0494
D12.0	32.3	15.3	0.91	130.	8.9	48.3	0.9840	6.1	56.8	10.09	0.8000	0.2164	0.0486	0.1512
D12.0	45.7	17.0	0.93	125.	8.5	43.9	0.9650	7.0	51.4	10.75	0.8020	0.2487	0.0569	0.1848
	MEANS		0.99	108.	9.5	51.0	0.9729	5.6	73.6	9.64	0.7941	0.2003	0.0540	0.1363

DATA FROM INDIVIDUAL FISH

MEANS ARE GIVEN AT THE BOTTOM OF EACH TEN FISH

U=RISING PHASE D=FALLING PHASE C=CONTROL FISH

TEMP	WT.	LTH.	C.C.	NA P	K P	CL P	H2O P	NA T	K T	CL T	H2O T	CL SP	NA SP	CLK SP
D10.0	43.2	16.0	1.05	100.	6.5	56.8	0.9830	4.0	92.1	8.72	0.7950	0.1589	0.0414	0.1283
D10.0	59.5	17.8	1.05	116.	6.7	72.9	0.9720	5.9	62.2	8.63	0.7990	0.1211	0.0520	0.0638
D10.0	38.6	15.6	1.02	85.	6.2	92.9	0.9770	6.5	59.9	9.92	0.7940	0.1098	0.0786	0.0545
D10.0	31.0	14.5	1.02	116.	8.2	101.3	0.9730	6.4	72.1	10.07	0.7870	0.1018	0.0565	0.0395
D10.0	24.4	13.4	1.01	100.	7.3	62.5	0.9650	6.3	59.5	9.80	0.7930	0.1593	0.0640	0.1007
D10.0	25.5	13.4	1.04	130.	7.8	75.2	0.9780	6.5	52.6	8.78	0.7950	0.1202	0.0515	0.0370
D10.0	26.5	14.0	0.96	105.	5.5	83.3	0.9900	7.5	59.0	10.02	0.7870	0.1254	0.0744	0.0804
D10.0	34.0	15.6	0.90	115.	6.8	70.8	0.9850	6.3	66.1	8.89	0.8000	0.1302	0.0568	0.0781
D10.0	29.1	14.5	0.95	116.	5.2	62.2	0.9830	6.8	60.0	8.97	0.7880	0.1492	0.0607	0.1104
D10.0	28.5	14.1	1.01	110.	7.8	59.7	0.9700	6.8	54.5	9.27	0.7920	0.1585	0.0631	0.0883
	MEANS		1.00	109.	6.8	73.8	0.9776	6.3	63.8	9.31	0.7930	0.1334	0.0599	0.0781
C 8.0	71.0	18.6	1.10	75.	6.4	50.8	0.9700	4.9	48.3	7.84	0.7880	0.1576	0.0667	0.0944
C 8.0	101.8	21.5	1.02	93.	6.7	62.5	0.9590	5.3	50.1	8.27	0.7890	0.1336	0.0575	0.0632
C 8.0	68.2	19.0	0.99	86.	6.4	51.6	0.9820	7.2	48.7	7.19	0.8040	0.1440	0.0865	0.0760
C 8.0	51.2	17.0	1.04	100.	6.8	58.9	0.9550	6.9	52.5	8.83	0.7850	0.1507	0.0694	0.0873
C 8.0	71.2	19.4	0.98	75.	6.2	72.0	0.9640	7.6	49.3	11.60	0.7820	0.1635	0.1028	0.1061
C 8.0	75.3	19.0	1.10	100.	6.3	106.7	0.9600	7.1	48.9	8.75	0.7820	0.0829	0.0717	0.0060
C 8.0	58.6	17.2	1.15	96.	6.4	72.7	0.9500	6.8	59.6	10.41	0.7850	0.1432	0.0708	0.0910
C 8.0	67.4	18.2	1.11	100.	6.7	75.3	0.9770	8.2	48.1	9.53	0.7880	0.1302	0.0843	0.0573
C 8.0	84.4	17.2	0.95	104.	6.4	49.7	0.9500	6.8	57.7	8.40	0.7860	0.1690	0.0654	0.1190
C 8.0	32.3	14.7	1.02	104.	6.9	74.5	0.9570	7.6	49.2	9.29	0.7870	0.1256	0.0736	0.0493
	MEANS		1.05	93.	6.5	67.5	0.9624	6.8	51.2	9.01	0.7876	0.1400	0.0749	0.0750
C10.0	70.4	17.8	1.25	90.	5.2	46.3	0.9730	7.4	55.2	8.42	0.7930	0.1863	0.0842	0.1474
C10.0	60.7	17.5	1.13	90.	5.4	72.3	0.9770	5.8	54.3	8.87	0.7960	0.1262	0.0663	0.0756
C10.0	74.3	19.0	1.08	104.	6.4	93.2	0.9650	6.9	53.3	9.04	0.7970	0.0985	0.0674	0.0285
C10.0	53.0	16.5	1.18	111.	5.2	113.3	0.9570	5.8	52.2	8.36	0.7920	0.0743	0.0526	0.0145
C10.0	42.9	16.5	0.96	115.	6.3	97.4	0.9700	5.9	64.6	10.18	0.7950	0.1067	0.0524	0.0540
C10.0	71.8	18.5	1.13	108.	6.5	57.4	0.9550	6.2	53.2	13.50	0.7990	0.2364	0.0577	0.1903
C10.0	68.1	17.8	1.19	86.	6.7	59.7	0.9640	6.1	46.5	10.21	0.7940	0.1735	0.0720	0.1051
C10.0	68.8	18.6	1.07	108.	5.7	99.7	0.9580	5.6	46.3	8.78	0.7940	0.0888	0.0523	0.0145
C10.0	51.7	16.8	1.09	119.	6.2	58.2	0.9600	6.1	44.0	9.28	0.7950	0.1611	0.0518	0.0912
C10.0	48.9	16.0	1.19	123.	5.8	102.9	0.9510	5.6	45.6	8.80	0.7940	0.0856	0.0456	0.0068
	MEANS		1.13	105.	5.9	80.0	0.9630	6.1	51.5	9.54	0.7949	0.1338	0.0602	0.0728

DATA FROM INDIVIDUAL FISH

MEANS ARE GIVEN AT THE BOTTOM OF EACH TEN FISH

U=RISING PHASE D=FALLING PHASE C=CONTROL FISH

TEMP	WT.	LTH.	C.C.	NA P	K P	CL P	H2O P	NA T	K T	CL T	H2O T	CL SP	NA SP	CLK SP
C12.0	71.7	19.1	1.03	131.	4.9	128.8	0.9630	7.6	58.5	9.70	0.7950	0.0763	0.0588	0.0276
C12.0	58.1	17.3	1.12	136.	4.8	99.4	0.9500	6.3	59.1	8.27	0.7950	0.0832	0.0463	0.0363
C12.0	48.3	17.2	0.95	115.	4.8	113.7	0.9680	7.2	61.0	8.31	0.8000	0.0745	0.0638	0.0284
C12.0	56.7	17.2	1.11	119.	5.6	120.1	0.9670	6.7	63.0	8.96	0.7920	0.0759	0.0573	0.0244
C12.0	70.4	18.7	1.08	131.	4.5	118.9	0.9750	6.4	59.9	8.49	0.7970	0.0733	0.0501	0.0301
C12.0	61.2	17.6	1.12	108.	5.2	126.5	0.9740	5.6	69.4	8.97	0.8000	0.0727	0.0532	0.0292
C12.0	56.3	17.2	1.11	131.	4.5	98.0	0.9740	6.9	53.1	8.89	0.7990	0.0930	0.0540	0.0460
C12.0	56.2	17.2	1.11	140.	4.4	110.4	0.9720	6.5	67.9	9.08	0.7910	0.0842	0.0475	0.0492
C12.0	50.5	16.4	1.14	123.	5.5	115.5	0.9480	6.4	50.1	8.39	0.7930	0.0725	0.0519	0.0040
C12.0	59.7	17.8	1.06	96.	6.0	65.4	0.9770	7.7	52.1	10.23	0.8060	0.1609	0.0825	0.1052
	MEANS		1.08	123.	5.0	109.7	0.9668	6.7	59.4	8.93	0.7968	0.0866	0.0565	0.0380
C14.0	98.7	20.6	1.13	95.	4.6	115.2	0.9730	5.6	52.9	9.24	0.7980	0.0822	0.0604	0.0322
C14.0	57.5	18.0	0.99	108.	4.5	118.1	0.9800	8.0	51.3	8.86	0.7930	0.0774	0.0764	0.0274
C14.0	53.2	17.1	1.06	127.	5.4	119.2	0.9620	5.0	73.4	8.54	0.7840	0.0725	0.0399	0.0313
C14.0	47.1	16.5	1.05	131.	4.9	115.1	0.9650	5.0	75.7	9.22	0.7990	0.0814	0.0388	0.0450
C14.0	74.3	19.1	1.07	123.	5.1	121.2	0.9620	5.7	59.6	8.57	0.7840	0.0716	0.0469	0.0225
C14.0	51.9	17.4	0.98	140.	4.7	103.3	0.9720	6.8	59.5	8.76	0.7880	0.0868	0.0497	0.0439
C14.0	43.1	16.3	1.00	131.	5.3	121.6	0.9600	5.2	74.1	8.12	0.7900	0.0675	0.0401	0.0261
C14.0	37.1	15.0	1.09	111.	5.5	97.5	0.9540	5.7	61.4	9.33	0.7900	0.0961	0.0516	0.0466
C14.0	47.0	16.2	1.10	111.	5.8	104.5	0.9550	6.8	53.7	7.64	0.7930	0.0735	0.0616	0.0071
C14.0	26.4	14.0	0.96	167.	5.4	76.1	0.9700	7.5	69.4	8.84	0.8100	0.1186	0.0459	0.0775
	MEANS		1.04	124.	5.1	109.2	0.9653	6.1	63.1	8.71	0.7929	0.0828	0.0511	0.0360
C16.0	123.6	22.0	1.16	154.	4.3	101.6	0.9700	6.9	52.0	8.78	0.7610	0.0882	0.0457	0.0468
C16.0	93.6	19.6	1.24	154.	3.6	102.9	0.9650	7.3	65.1	11.56	0.7240	0.1141	0.0482	0.0923
C16.0	68.9	19.0	1.01	154.	4.4	97.9	0.9800	5.5	63.1	7.86	0.7820	0.0828	0.0368	0.0461
C16.0	64.2	17.5	1.20	115.	4.7	116.5	0.9460	6.0	70.0	8.40	0.7630	0.0718	0.0520	0.0361
C16.0	85.1	19.0	1.24	131.	4.1	118.6	0.9630	4.8	60.0	8.51	0.7890	0.0727	0.0371	0.0342
C16.0	66.3	18.0	1.14	115.	4.3	58.1	0.9490	7.6	52.2	10.33	0.7750	0.1776	0.0660	0.1444
C16.0	71.5	18.2	1.19	150.	4.0	103.6	0.9550	7.6	64.9	8.85	0.7580	0.0859	0.0509	0.0555
C16.0	98.1	19.4	1.34	111.	3.9	93.8	0.9420	6.1	54.8	7.96	0.7730	0.0841	0.0545	0.0461
C16.0	48.3	15.7	1.25	136.	4.4	68.9	0.9710	6.6	62.8	7.60	0.7760	0.1127	0.0496	0.0793
C16.0	36.6	15.0	1.08	117.	4.0	84.1	0.9530	5.8	61.9	8.08	0.7780	0.0964	0.0497	0.0633
	MEANS		1.18	134.	4.2	94.6	0.9594	6.4	60.7	8.79	0.7679	0.0986	0.0491	0.0644

DATA FROM INDIVIDUAL FISH

MEANS ARE GIVEN AT THE BOTTOM OF EACH TEN FISH

U=RISING PHASE D=FALLING PHASE C=CONTROL FISH

TEMP	WT.	LTH.	C.C.	NA P	K P	CL P	H2O P	NA T	K T	CL T	H2O T	CL SP	NA SP	CLK SP
C18.0	71.5	18.1	1.21	123.	4.1	83.8	0.9730	6.9	49.0	10.35	0.7720	0.1265	0.0575	0.0880
C18.0	102.8	20.0	1.29	123.	3.9	82.3	0.9300	6.3	43.2	8.93	0.7730	0.1062	0.0501	0.0589
C18.0	80.9	18.2	1.34	127.	4.0	59.9	0.9550	4.1	67.6	8.59	0.7700	0.1442	0.0325	0.1190
C18.0	66.9	17.6	1.23	170.	3.9	96.7	0.9720	5.0	82.4	10.99	0.7720	0.1163	0.0301	0.0950
C18.0	76.7	18.7	1.18	127.	4.7	128.1	0.9600	5.7	52.2	8.42	0.7880	0.0664	0.0454	0.0128
C18.0	93.6	19.5	1.26	158.	4.0	86.3	0.9480	6.4	48.8	8.58	0.7790	0.0992	0.0404	0.0561
C18.0	87.7	18.0	1.15	119.	4.0	98.4	0.9640	8.8	48.2	11.49	0.7780	0.1185	0.0750	0.0782
C18.0	106.8	20.6	1.22	115.	4.0	112.4	0.9700	8.0	46.8	9.26	0.7820	0.0841	0.0710	0.0376
C18.0	68.1	18.7	1.04	145.	4.6	100.3	0.9530	8.7	49.9	10.53	0.7580	0.1053	0.0602	0.0603
C18.0	82.3	19.0	1.20	167.	3.9	86.0	0.9650	7.3	47.9	10.97	0.7780	0.1296	0.0444	0.0915
	MEANS		1.21	137.	4.1	93.4	0.9590	6.7	53.6	9.81	0.7750	0.1096	0.0507	0.0698

APPENDIX III

Correction curves to correct for potassium background on the sodium signal.